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# Uptake of Waterborne Methylmercury by Rainbow Trout (*Salmo gairdneri*) in Relation to Oxygen Consumption and Methylmercury Concentration

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RODGERS, D. W., AND F. W. H. BEAMISH. 1981. Uptake of waterborne methylmercury by rainbow trout (*Salmo gairdneri*) in relation to oxygen consumption and methylmercury concentration. Can. J. Fish. Aquat. Sci. 38: 1309–1315.

Oxygen consumption and uptake of waterborne methylmercury were measured for rainbow trout (*Salmo gairdneri*) forced to swim at sustained swimming speeds at 10 and 20°C. The concentrations of methylmercury used (< 8 μg $Hg \cdot L^{-1}$) did not affect oxygen consumption. The log of mass specific oxygen consumption increased linearly with relative swimming speed. Standard metabolic rates were significantly different ($P < 0.05$) at 10 and 20°C (111 and 192 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$, respectively) but the rate of increase in oxygen consumption with swimming speed was not significantly different between temperatures. The rate of methylmercury uptake was positively correlated with both oxygen consumption and methylmercury concentration. Multiple linear regression equations relating the logs of rate of methylmercury uptake, rate of oxygen consumption, and methylmercury concentration at 10 and 20°C were contiguous and a single equation described the relationship at both temperatures. The efficiency of methylmercury uptake relative to oxygen was ~0.25 at both temperatures. Assuming an oxygen percent utilization of 33%, the percent utilization of methylmercury from water was ~8%.

*Key words*: methylmercury, uptake, respiration, oxygen consumption, rainbow trout, bioaccumulation, pollutant

RODGERS, D. W., AND F. W. H. BEAMISH. 1981. Uptake of waterborne methylmercury by rainbow trout (*Salmo gairdneri*) in relation to oxygen consumption and methylmercury concentration. Can. J. Fish. Aquat. Sci. 38: 1309–1315.

Chez des truites arc-en-ciel (*Salmo gairdneri*) forcées à une nage soutenue à 10 et 20°C, nous avons mesuré la consommation d'oxygène et l'absorption de mercure-méthyl. Les concentrations de mercure-méthyl utilisées (<8 μg $Hg \cdot L^{-1}$) n'influencent pas la consommation d'oxygène. Le log de la consommation d'oxygène par rapport à la masse augmente en fonction linéaire de la vitesse de nage relative. Les taux métaboliques normaux sont significativement ($P < 0,05$) différents à 10 et 20°C (111 et 192 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$, respectivement), mais le taux d'augmentation de la consommation d'oxygène en fonction de la

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vitesse de nage ne diffère pas de façon significative à ces deux températures. Il y a corrélation positive entre le taux d'absorption de mercure-méthyl, d'une part, et la consommation d'oxygène et la concentration de mercure-méthyl, d'autre part. Les équations de régressions linéaires multiples reliant les logs du taux d'absorption du mercure-méthyl, les taux de consommation d'oxygène et la concentration de mercure-méthyl à 10 et 20°C sont contiguës, et une seule équation décrit la relation à l'une et l'autre température. L'efficacité d'absorption du mercure-méthyl en fonction de l'oxygène est ~0,25 aux deux températures. Si l'on suppose une utilisation de l'oxygène de 33%, le pourcentage d'utilisation du mercure-méthyl de l'eau est environ 8%.

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THE gills of teleost fishes are central to the exchange of dissolved gases, ions, and organic molecules (Conte 1969; Foster and Goldstein 1969; Randall 1970). The mechanisms of exchange may range from passive, including exchange and facilitated diffusion, to active carrier-mediated transport. Passive fluxes across the gills are governed by chemical and electrical gradients and by the physical properties of the substance exchanged (Hunn and Allen 1974). In particular, lipid soluble molecules may be absorbed selectively from solution by surface absorption and partitioning to the lipoprotein cell membrane (Bryan 1979). Both active and passive exchange are affected by the volume of water passed over the gills, which is determined by metabolic rate (Jones and Randall 1978).

Lipophilic and amphyphilic molecules once across the gills are transported by the circulatory system to other tissues (Olsen et al. 1973). The distribution of these compounds reflects their affinity to specific tissues and the pattern of circulation, while the concentration of the compound attained by the tissue depends on both distribution and the rate of metabolism or elimination from the tissue (deFreitas and Norstrom 1974). For compounds that are only slowly metabolized or eliminated from the fish, tissue concentrations substantially above ambient may be accumulated. The concentrations of many xenobiotics such as DDT, PCBs, and methylmercury attained by fish in contaminated waters (Hamelink et al. 1977; Norstrom et al. 1976) are examples of this accumulation process.

Numerous studies have demonstrated that fish readily accumulate waterborne xenobiotics which themselves may interfere with respiration (Hughes 1973). However, relatively few have attempted to relate uptake of these compounds to respiratory rate. The potentiation of the toxicity of a number of compounds to rainbow trout by low dissolved oxygen concentration was attributed to a coincident increase in respiratory volumes (Lloyd 1961). Both oxygen consumption and uptake of waterborne DDT increased with body weight to a similar exponent in mosquito fish, *Gambusia affinis* (Murphy and Murphy 1971). More recently, the uptake of waterborne xenobiotics has been related directly to oxygen consumption in restrained salmonids (Boddington et al. 1979; McKim 1981).

Our experiments were designed to test the relationships among oxygen consumption, methylmercury concentration, and uptake in rainbow trout, *Salmo gairdneri*, over a broad range of metabolic rates achieved by forcing the fish to swim at speeds between low-sustained and near-critical velocities at two temperatures.

## Materials and Methods

Rainbow trout (4–10 g) were purchased from a private hatchery (Goosens, Otterville, Ont.), tagged (Floy fingerling tags – Floy Tag Manufacturing Ltd., Seattle, WA), and held in 50-L circular troughs supplied with aerated well water (11 ± 1.0°C). A submersible pump installed in each trough created a current of ~8 cm/s. Fish were acclimated to either 10 or 20°C by adjusting temperature at the rate of 1°C/d, then maintained at that temperature (±0.5°C) for at least 2 wk prior to an experiment. Conductivity, total hardness, and pH of the well water ranged from 0.64 to 0.72 mS/cm, 375 to 390 mg/L, and 8.0 to 8.2, respectively (APHA et al. 1971).

Fish were fed a commercial trout diet (GRT-78G–Martins Feed Mills, Elora, Ont.) once every 2nd day at a meal size equivalent to 2% wet body weight. Photoperiod was maintained at 16 h of light with a gradual change of intensity for $\frac{1}{2}$ h at each end of the light regime.

Individual fish were transferred from a holding tank to a modified Blazka-type respirometer (Waiwood and Beamish 1978) at ~18:00 the day before an experiment. Aerated well water from a temperature-regulated head tank flowed to each respirometer at a rate of ~150 mL/min. The respirometers were submerged in a water bath, with both bath and head tank maintained at the acclimation temperature of the fish. Fish were required to swim at ~10 $cm \cdot s^{-1}$ (1.2 body lengths $(BL) \cdot s^{-1}$) overnight. At 09:00 the next day, the water velocity was increased over a 5-min period to either 20, 30, or 40 $cm \cdot s^{-1}$ and maintained at that speed throughout the experiment. These velocities were selected to provide a broad range of aerobic metabolic rates with the highest velocity equivalent to ~80% of critical speed (Waiwood and Beamish 1978). One hour after speed adjustment, dissolved oxygen concentration of the outflow of water of the respirometer was determined and the inflow stopped for an interval of 1 or 2 h at 20 and 10°C, respectively. Dissolved oxygen concentration was measured with an oxygen electrode and analyser (YSI, model 54) calibrated daily with the sodium azide modification of the Winkler method (APHA et al. 1971). The maximal cross-sectional area of the trout as determined from the relationship of cross-sectional area and weight of fish of the same stock (Waiwood 1977) was less than 10% of the cross-sectional area of the swimming chamber. As speed corrections should be small enough to ignore for fish occupying less than 10% of the area of the swimming chamber (Webb 1975), measured water velocities were used without correction.

At the end of the interval, water flow through the respirom-

eter was resumed and the dissolved oxygen concentration of the outflow determined. Throughout all tests, dissolved oxygen concentrations remained above 70% of air saturation. The difference in oxygen content between the beginning and end of the test interval, minus the aerobic demands of the chamber water in the absence of fish, provided the measurement of oxygen consumption.

Oxygen consumption of each fish was determined for three intervals. Between determinations, the flow of aerated water through the chamber was resumed for 1 h to allow dissolved oxygen to return to at least 90% of air saturation. Immediately preceeding the third interval, [$^{203}$Hg]methylmercury (0.5–2.5 mL) was injected into the inflow water 1 min before the system was closed. Duplicate water samples (5 mL) were collected before injection, at 0 and 10 min and at the end of the time interval for determination of methylmercury concentration. At the end of the third interval, the fish was immediately removed from the respirometer and transferred to a holding tank. Within 24 h of the experiment, the fish was weighed and measured to the nearest 0.1 g and mm, respectively, and the uptake of methylmercury determined by whole body gamma counting. As the biological half-life of methylmercury in fish of this size is greater than 50 d (deFreitas et al. 1975), a correction for elimination before counting was not required.

Methylmercuric chloride labeled with $^{203}$Hg (specific activity 17.5–87.5 MBq/mg Hg) (New England Nuclear, Montreal, P.Q.) was maintained as an aqueous solution at a concentration of ~0.4 MBq·mL$^{-1}$. At this concentration the level of $^{203}$Hg-labeled impurities remained at less than 4% as measured by extraction with acidified benzene (Westoo 1973).

Methylmercury in water and fish was measured by gamma spectrometry of the [$^{203}$Hg]methylmercury. Water samples were counted by an automated gamma counter with a 7.5-cm-diameter, 7.5-cm-high, well-shaped NaI(T1) crystal (Nuclear Chicago model 4233). Fish were counted in a manual whole body counter with two 5 × 5 × 15 cm NaI(T1) crystals as the detection unit. The rectangular faces of the crystals were horizontally opposed with a 5-cm space between crystals. The detection unit was shielded with lead bricks and the output of the two crystals summed and connected to a preamplifier, amplifier, single channel analyser, scaler, and timer (Harshaw detectors and electronics).

Fish were centered in a cylindrical acrylic container filled with uncontaminated water. The container was closed and placed in a fixed groove with the fish midway between the NaI(T1) crystals. Three 1-min counts were made of each fish: fish counted at greater than 3000 cpm (background less than 250 cpm) and replicate counts varied by less than 3%. In a preliminary study, counting efficiency was not affected by fish size for fish from less than 5 to 50 g. Phantoms containing known quantities of nuclide were prepared from each batch of isotope and used as internal standards to correct for radioactive decay and interrelate counts on the deep well and whole body counters. Results, corrected for background, counting efficiency, and radioactive decay are expressed as nanograms of methylmercury.

The concentration of [$^{203}$Hg]methylmercury in a closed respriometer, without fish, decreased rapidly (Fig. 1). This loss was apparently due to adsorption and absorption to the acrylic walls of the respirometer. An exponential decay function adequately described this loss with correlation coefficients of 0.96 or greater obtained in individual linear regressions of log concentration of isotope and time (14 trials, samples taken as indicated in Fig. 1). It was assumed that a similar exponential decay function would apply to the decrease in nuclide concentration in experiments with a fish in the respirometer as methylmercury uptake by the fish constituted less than 3% of the quantity of isotope introduced. The concentration of methylmercury experienced by the fish was thus determined as the mean of the logarithm of concentrations of [$^{203}$Hg]methylmercury at the beginning and end of exposure. Initial concentrations of methylmercury were in the range of 1–10 μg $CH_3Hg \cdot L^{-1}$ with average concentrations from 0.5 to 4.0 μg $CH_3Hg \cdot L^{-1}$. Water samples taken before injection of nuclide were counted at background rates as did fish held in the respirometers but not exposed to isotope.

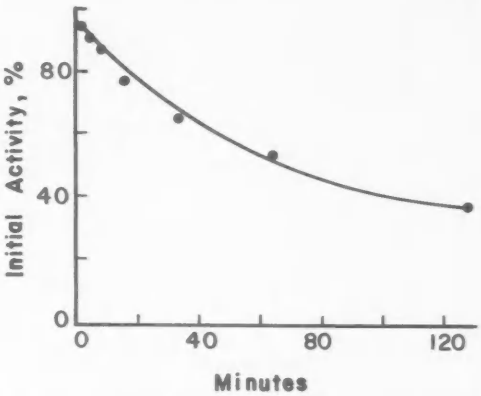


FIG. 1. Decrease in concentration of [$^{203}$Hg]methylmercury in water from a closed respirometer during a 128-min period (mean of 14 trials).

The efficiency of methylmercury uptake relative to oxygen ($E(p/o)$) was estimated as:

$$E(p/o) = (\Delta P/Cpw)/(Q/[O_2])$$

where $\Delta P$ is the rate of methylmercury uptake of the fish (ng $CH_3Hg \cdot h^{-1}$), Cpw is the methylmercury concentration (ng $CH_3Hg \cdot mL^{-1}$), $Q$ is the rate of oxygen consumption of the fish (mg $O_2 \cdot h^{-1}$) and $[O_2]$ is the mean concentration of oxygen (mg $O_2 \cdot mL^{-1}$).

Comparisons between means were made using appropriate $T$-tests at $P < 0.05$. Least squares regressions were compared using either large sample $Z$ tests or dummy variables (Kleinbaum and Kupper 1978). All regressions were significant at $P < 0.05$. The log transformation was used as indicated by residual plots.

## Results

Oxygen consumption of rainbow trout was not affected by methylmercury at the concentrations and durations of exposure used. At both 10 and 20°C, the rate of oxygen con-

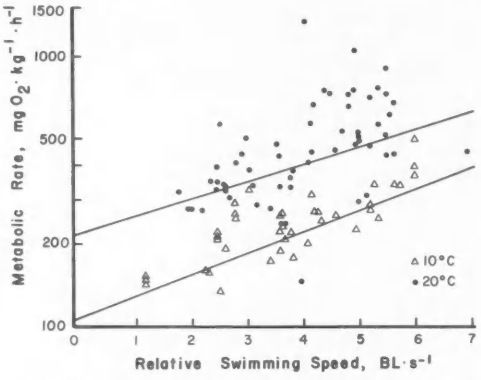


FIG. 2. Increase in mass specific oxygen consumption with a relative swimming speed of ~6.5-g rainbow trout at 10 and 20°C.

sumption for the interval in which methylmercury was added was not signficantly different from the preceeding interval ($P < 0.05$).

The mass specific rate of oxygen consumption increased with relative swimming speed at both 10 and 20°C with values obtained for individual fish shown in Fig. 2. The relationships are described by the equations:

(1) 10°C $\quad \text{Log } M = 2.030 + 0.082\ S$

$(R^2 = 0.60;\ n = 38)$

(2) 20°C $\quad \text{Log } M = 2.330 + 0.068\ S$

$(R^2 = 0.25;\ n = 60)$

where $M$ is the mass specific rate of oxygen consumption (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) and $S$ is the relative swimming speed ($BL \cdot s^{-1}$). Fish swam well at all speeds with no obvious differences in swimming behavior between temperatures. The mean size of fish was similar at both temperatures although fish at 20°C were somewhat more variable (6.7 ± 0.28 g (mean ± SE) and 76 ± 1.0 mm at 10°C; 6.3 ± 0.36 g and 78 ± 1.3 mm at 20°C).

The increased variability of metabolic rate of fish at 20°C is also apparent in the lower $R^2$ of equation (2). The rate of increase in oxygen consumption with swimming speed was not significantly different ($P < 0.05$) at 10 and 20°C. However, standard metabolic rates, calculated by extrapolation of the linear regressions to zero swimming speed (Brett 1965), were significantly different ($P < 0.05$) at 10 and 20°C (107 and 214 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 10 and 20°C, respectively), thus equations (1) and (2) were distinct. At swimming speeds of 6 $BL \cdot s^{-1}$, approximately critical speed for fish of this size (Waiwood and Beamish 1978), calculated metabolic rates are 332 and 545 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 10° and 20°C, respectively. The increase from standard to active metabolic rates or metabolic scope (Fry 1947) of the fish was 225 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 10° and 331 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 20°C.

The rate of methylmercury uptake was positively related to both rate of oxygen consumption and methylmercury concentration as seen in Fig. 3 and 4, respectively. The multiple linear regression equations describing the relationship among these variables at each temperature are:

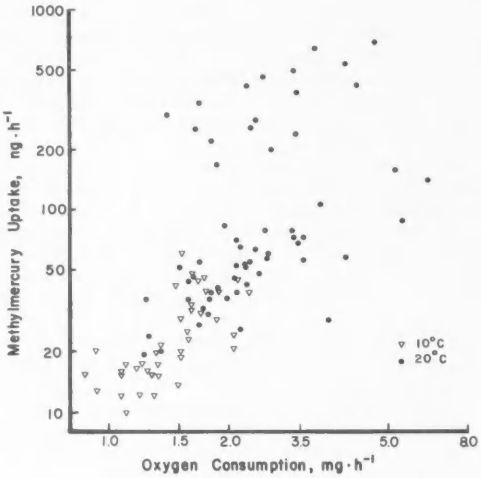


FIG. 3. Effect of oxygen consumption on the rate of methylmercury uptake of rainbow trout at 10 and 20°C.

(3) 10°C $\quad \text{Log } \Delta P = 1.326 + 1.147 \log Q + 0.724 \log Cpw$

(multiple $R^2 = 0.74;\ n = 38$)

(4) 20°C $\quad \text{Log } \Delta P = 1.479 + 1.106 \log Q + 1.197 \log Cpw$

(multiple $R^2 = 0.88;\ n = 60$)

where $\Delta P$, $Q$, and $Cpw$ are as defined earlier.

The regression equations at each temperature are contiguous and can be reduced to a common regression which is not significantly different from the individual regressions ($P < 0.05$), yet had a higher $R^2$ than regressions at each temperature. The regression equation describing the pooled data is:

(5) $\quad \text{Log } \Delta P = 1.457 + 1.097 \log Q + 1.152 \log Cpw$

(multiple $R^2 = 0.91;\ n = 98$).

In equations (3), (4), and (5), both rate of oxygen consumption and methylmercury concentrations are highly significant factors ($P < 0.001$) in the prediction of rate of methylmercury uptake. Further, the dependent variables are largely independent in their contribution to the regression, with the variability ($R^2$) of regressions using either variable by itself approximately one-half the multiple $R^2$ using both variables.

Equation (5) could be further simplified by replacing the terms log $Q$ and log $Cpw$ with a combined term log ($Q \times Cpw$) as the coefficients of the above terms were not signifi-

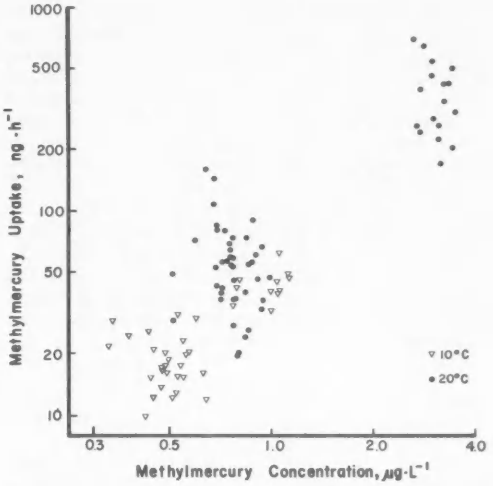


FIG. 4. Effect of methylmercury concentration on the rate of methylmercury uptake of rainbow trout at 10 and 20°C.

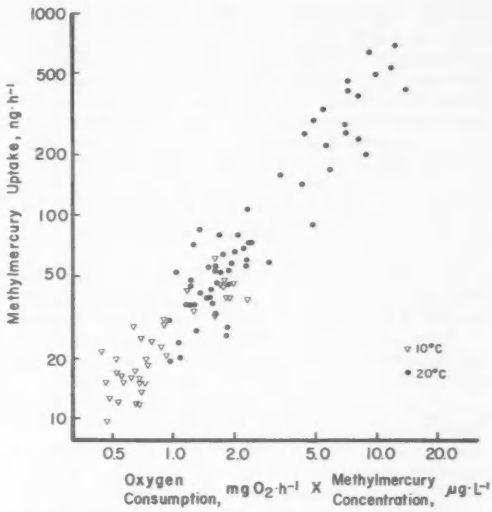


FIG. 5. Relationship of methylmercury uptake rate to the product of oxygen consumption and methylmercury concentration for rainbow trout at 10 and 20°C.

cantly different ($P < 0.05$). The relationship between log $\Delta P$ and log ($Q \times Cpw$) for individual fish at both temperatures is illustrated in Fig. 5. The regression equation describing this relationship is:

$$\text{(6)} \quad \text{Log } \Delta P = 1.445 + 1.134 \log (Q \times Cpw)$$

$$(R^2 = 0.91;\ n = 98).$$

The efficiency of methylmercury uptake relative to oxygen was not significantly different ($P < 0.05$) at 10 and 20°C with an overall mean of ~0.25 (0.24 ± 0.01 (mean ± SE) at 10°C and 0.26 ± 0.01 at 20°C). This ratio was essentially independent of the rates of oxygen consumption and methylmercury uptake observed and the concentrations of oxygen and methylmercury used. Simple correlation coefficients between $E(p/o)$ and any of the above variables were less than 0.55.

## Discussion

The concentrations (<5.0 µg $Hg \cdot L^{-1}$) and durations of exposure to methylmercury we used were well below acutely toxic levels and did not affect rainbow trout as measured by effects on oxygen consumption. This is consistent with the observation that brook trout, *Salvelinus fontinalis*, exposed to methylmercury concentrations of less than 3 µg $Hg \cdot L^{-1}$ showed no indication of stress as measured by cough frequency (Drummund et al. 1974). The 96-h LC50's of methylmercury to rainbow trout and brook trout are reported as 31 and 75 µg $Hg \cdot L^{-1}$, respectively (Matida et al. 1971; McKim et al. 1976).

The rates of weight-specific oxygen consumption obtained at both 10 and 20°C are in agreement with metabolic rates previously reported for rainbow trout. The metabolic rates calculated from equations (1) and (2) for trout swimming at 6 BL/s, assuming a weight exponent of 1.0 (Rao 1968) (332 and 545 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 10 and 20°C, respectively), fall within the limits of active metabolism reported by Dickson and Krammer (1971) for two strains of rainbow trout (468–492 and 570–592 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 10 and 20°C, respectively). Using a weight exponent of 0.8 for standard metabolic rate (Rao 1968), the standard metabolic rates of 157 and 309 mg $O_2 \cdot kg^{-0.8} \cdot h^{-1}$ at 10 and 20°C for 6-g trout in this study are in agreement with standard metabolic rates of 143 and 278 mg $O_2 \cdot kg^{-0.8} \cdot h^{-1}$ reported for 100-g rainbow trout at 5 and 15°C (Rao 1968). The linear relationship between the log of weight-specific oxygen consumption and swimming speed is consistent with the pattern reported for this and other salmonids (Brett 1965; Rao 1968; Waiwood and Beamish 1978). The greater variability at 20°C may reflect the greater variability in size of fish tested at this temperature, or that fish at 20°C were stressed by temperature. Although the upper incipient lethal temperature for rainbow trout is 25.6°C, the optimum temperature for growth and yield is in the range of 17°C (Hokanson et al. 1977). In these growth experiments, at temperatures above optimum, mortality rates increased significantly (Hokanson et al. 1977), suggesting that at elevated sublethal temperatures the trout are stressed. A similar increase in the variability of regressions between weight-specific rate of oxygen consumption and swimming speed has been reported for rainbow trout stressed by sublethal concentrations of copper or rotenone (Waiwood and Beamish 1978; Skadsen et al. 1980).

We have demonstrated that the rate of uptake of waterborne methylmercury may be accurately described from oxygen consumption and methylmercury concentration. Our finding that regression equations relating methylmercury uptake, oxygen consumption, and methylmercury concentration at 10

and 20°C could be combined into a single equation substantiates the proposition of deFreitas and Hart (1975) that temperature affects methylmercury uptake by a direct effect on metabolic rate. It is probable that many previous reports of temperature effects on xenobiotic uptake (Murphy and Murphy 1971; Reinert et al. 1974) can be similarly explained. It is also apparent that in studies of xenobiotic accumulation, factors which affect metabolic rate such as handling and feeding may markedly affect the results obtained. As the toxicity of xenobiotics may relate to rates of uptake (Roberts et al. 1978), similar considerations may also apply to toxicity studies.

The relationship between concentration of methylmercury and rate of methylmercury uptake was linear for a duration of at least 24 d and was not affected by simultaneous expsoure to dietary methylmercury (Phillips and Buhler 1978). Thus equation (6) should be valid for predicting long-term uptake of waterborne methylmercury.

The consistency of the efficiencies of uptake of methylmercury relative to oxygen at both temperatures suggests that this ratio (0.25) may be characteristic of the compound and experimental conditions used. The percent utilization of oxygen from water for rainbow trout swimming in well-aerated water was a constant value of 33%, at swimming speeds from less than 20% of critical to critical (Kiceniuk and Jones 1977). The percent utilization of methylmercury from water calculated using the value of 33% is 8.3%; a value consistent with a percent utilization of methylmercury of 7.1% reported by Boddington et al. (1979). Under conditions of oxygen stress imposed by low concentrations of dissolved oxygen, the percent utilization of oxygen from the water may decrease (Kerstens et al. 1979; McKim 1981). If the efficiency of uptake of pollutant relative to oxygen remains stable, the percent utilization of pollutant should decrease as well, which is consistent with the observations of McKim (1981) on the effects of low concentrations of dissolved oxygen on endrin uptake in brook trout.

Equations (3), (4), (5), and (6) do not require assumptions of the efficiencies of removal of methylmercury and oxygen from the water and thus represent a significant simplification over the expressions used by Norstrom et al. (1976) to describe methylmercury uptake from water. For our data, the replacement of the rate of oxygen consumption by the rate of oxygen consumption divided by mean oxygen concentration did not improve predictions of rate of methylmercury uptake, and the simpler model was selected.

A number of water quality factors such as pH, alkalinity, and the presence of selenium have been reported to affect methylmercury accumulation in wild fish (Scheider et al. 1979; Speyer 1980). These factors could affect accumulation either through effects on the levels or distribution of methylmercury (Miller and Akagi 1979) or through effects on uptake and retention by the fish. An analysis of the effects of these entities on the efficiency of uptake of methylmercury relative to oxygen may be of particular use in identifying the mechanisms by which they affect accumulation. Conversely, comparisons of the efficiency of pollutant uptake relative to oxygen of different pollutants under similar conditions may provide a sensitive standard index of the potential for pollutants to be accumulated from water.

As our data define the relationship between methylmercury uptake, oxygen consumption, and methylmercury concentration with precision, a measure of two of the above can provide an estimate of the third. Thus if the rate of oxygen consumption of a fish is known or can be estimated, a measure of the rate of uptake of waterborne methylmercury of the fish will provide an estimate of methylmercury concentration (see also Fagerstrom and Asell 1976). Further, as low concentrations of [$^{203}$Hg]methylmercury, such as were used in this study, did not affect the oxygen consumption of the fish, but are easily measured, a measure of the rate of uptake of [$^{203}$Hg]methylmercury by a fish under defined exposure concentrations gives an estimate of the fish's metabolic rate. This measurement may be particularly valuable in quantifying metabolic rate in situations not ammenable to conventional techniques.

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# Radiotracer Experiments on Phosphorus Uptake and Release by Limnetic Microzooplankton

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TAYLOR, W. D., AND D. R. S. LEAN. 1981. Radiotracer experiments on phosphorus uptake and release by limnetic microzooplankton. Can. J. Fish. Aquat. Sci. 38: 1316–1321.

When carrier-free $^{32}PO_4$ is added to lakewater, the planktonic ciliated protozoan *Strombidium viride* gradually becomes labeled over at least 30 h. The label enters *S. viride* primarily by the ingestion of labeled particles 0.2–1.0 μm in size; direct uptake of phosphate or uptake of larger particles (≥5 μm) is relatively slight or absent. When labeled *S. viride* are transferred to unlabeled lake water, they lose $^{32}P$ by a discontinuous process, presumed to be defecation, plus a relatively slow, continuous process. The latter is assumed to be equivalent to what is usually termed excretion for crustacean zooplankton, and is equivalent to a turnover time for the animal's phosphorus content of many hours. Results of release experiments with the rotifer *Keratella cochlearis* are quantitatively similar, but defecation was a much larger part of the total. Excretion was similar in turnover time to that for *S. viride*. The soluble phosphorus released by both species was characterized as primarily phosphate using gel filtration. These results do not support the conventional view that microzooplankton regenerate dissolved phosphorus much more rapidly for their biomass than larger crustacean zooplankton.

*Key words*: phosphorus uptake, phosphorus release, microzooplankton, *Strombidium viride*, *Keratella cochlearis*

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Quand on ajoute à l'eau de lac $^{32}PO_4$ sans porteur, le protozoaire cilié planctonique *Strombidium viride* devient marqué pendant au moins 30 h. La marque pénètre *S. viride* surtout par ingestion de particules marquées de 0,2 à 1,0 μm; il n'y a que très peu ou pas du tout d'assimilation directe de phosphates ou d'ingestion de grosses particules (≥5 μm). Quand des *S. viride* marqués sont transférés dans de l'eau de lac non marquée, ils perdent le $^{32}P$ par un processus discontinu, qu'on suppose être la défécation, et par un processus continu relativement lent. On suppose que ce dernier équivaut à ce que l'on appelle ordinairement excrétion chez les crustacés planctoniques et correspondant à un temps de renouvellement de plusieurs heures du phosphore chez l'animal. Les résultant d'expériences de mise en liberté du phosphore avec le rotifère *Keratella cochlearis* sont quantitativement semblables, sauf que la défécation représente une proportion plus grande du total. L'excrétion est identique quant au temps de renouvellement à celui de *S. viride*. La filtration sur gel a permis de caractériser le phosphore soluble libéré par les deux espèces comme étant surtout des phosphates. Ces résultats ne s'accordent donc pas avec le point de vue conventionnel que le microzooplancton regénère le phosphore dissous beaucoup plus rapidement, compte tenu de sa biomasse, que les gros crustacés planctoniques.

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DURING the summer period of thermal stratification in lakes, epilimnetic phosphorus is exchanged rapidly between a small dissolved fraction and the biota (Rigler 1956; Lean 1973). Uptake of dissolved phosphate is by bacteria and small algae (Rigler 1956; Paerl and Lean 1976). The return of phosphorus to solution may be largely due to phytoplankton excretion (Lean and Nalewajko 1976; Lean and Charlton 1976), but release by consumers and inputs from outside the epilimnion must balance the net production of phytoplankton lost to herbivores and the sediment (Rigler 1963; Lehman 1980).

The importance of nutrient release by zooplankton, although widely accepted, is difficult to illustrate. Studies of phosphorus release by crustacean zooplankton usually docu-

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TABLE 1. Conditions at the Sharpe's Bay station during the June and September experiments. Quantities are expressed as μg/L unless labeled otherwise.

| Date | Depth (m) | Temp. (°C) | $O_2$ (mg/L) | Chlorophyll | Total phosphorus | Soluble reactive phosphorus | Particulate carbon |
|---|---|---|---|---|---|---|---|
| June 15 | 1 | 16.4 | 10.8 | — | — | — | — |
| | 2 | 16.4 | 10.8 | 2.4 | 17 | 1 | 369 |
| | 5 | 16.4 | 10.8 | 2.4 | 18 | 1 | 345 |
| | 10 | 8.6 | 11.4 | 2.7 | 18 | 1 | 404 |
| | 20 | 6.1 | 11.4 | 0.5 | 19 | 2 | 263 |
| Sept. 22 | 1 | 16.8 | 9.6 | — | — | — | — |
| | 2 | 16.8 | 9.6 | 3.1 | 12 | 1 | 780 |
| | 5 | 16.8 | 9.6 | 3.3 | 16 | 2 | 806 |
| | 10 | 10.1 | 5.1 | 0.9 | 13 | 1 | 807 |
| | 20 | 5.8 | 5.8 | 0.5 | 13 | 5 | 500 |

ment rather low rates of release (e.g. Rigler 1961; Lehman 1980), but results by different investigators vary widely (Peters and Rigler 1973). Nevertheless, it has been observed repeatedly that the rate of phosphorus release per unit mass is higher for smaller zooplankton (Johannes 1964; Barlow and Bishop 1965; Peters and Rigler 1973). This has led to speculation that very small zooplankton, particularly ciliated protozoa, might be particularly important in mineralization of limnetic phosphorus. Two laboratory studies (Johannes 1965; Buechler and Dillon 1974) have supported this view by finding rapid release of phosphorus by ciliates. Measured body equivalent excretion times (BEETS) were 5–160 min, depending on species, growth phase, temperature, etc. Most were near the low end of this range.

To investigate the importance of ciliates in mineralization, we elected to investigate phosphorus uptake and release by a ciliate species abundant in lake plankton, and performing the experiments in lake water rather than culture media. We chose *Strombidium viride*, an oligotrich ciliate with zoochlorellae, as the main focus of our work. It is larger than most planktonic ciliates (and therefore easier to handle) and it is common in lake plankton. To enable us to compare *S. viride* with a small metazoan zooplankter, we repeated our excretion experiments with the common rotifer *Keratella cochlearis*.

## Materials and Methods

The experiments were all conducted at Jack's Lake (44°41′ lat., 78°03′ long.) near Peterborough, Ont., during June 9–13 and September 22–26, 1980. We worked at the deepest point (43 m) in oligotrophic Sharpe Bay (530 ha). Tables 1 and 2 summarize conditions during the experiments.

For $^{32}P$ uptake experiments, unfiltered lake water was incubated in glass or nalgene containers. During June experiments, water was collected from 10 m and incubated at 5 m, while in September water was collected at 2 m and incubated at 1 m. The depths where the water was collected were those with the largest numbers of *S. viride*. The water was spiked with ~3.7 MBq/L of carrier-free $^{32}PO_4$. We spiked the lake water, rather than adding labeled food, because the feeding habitats of microzooplankton are largely unknown. At the same time, a smaller volume was spiked with about 10 times as much label per volume for use in excretion experiments. In September, a parallel sample was spiked and incubated in the laboratory to study uptake over the first hour, and duplicates of both were incubated with 10 μg P/L as phosphate added.

TABLE 2. Abundance (no./mL) and biomass (μg/L wet weight) for *S. viride* and other ciliates during the June and September experiments.

| Date | Depth | Total ciliates | | *S. viride* | |
|---|---|---|---|---|---|
| | | Number | Biomass | Number | Biomass |
| June 10 | 10 m | 19.0 | 83 | 2.8 | 47.0 |
| Sept. 23 | 2 m | 8.6 | 52 | 0.16 | 0.26 |

Filtrations were done with Nuclepore filters and 0.1 to 10 mL of sample, depending on the anticipated amount of label. They were dissolved in 1:10 ethanolamine in methylene chloride (Kent Burnison, N.W.R.I., Burlington, Ont. personal communication) before being counted in fluor (PCS, Amersham) on a scintillation counter.

Animals were collected for scintillation counting by picking them singly from the lake water with a micropipette made by drawing a Pasteur pipette in a flame. Prior to counting their degree of labeling with $^{32}P$, they were washed by passing them through three washes of 1-mL volume contained in 3-spot color reaction plates.

Excretion and feeding experiments were also conducted in 3-spot plates. These were kept at an appropriate temperature (that of the depth where the animals were collected) by placing them in plastic weighing boats floating in a temperature-controlled bath. For each excretion experiment, filtered (12 μm) lake water was placed in the depressions of several 3-spot plates. A single animal from lake water labeled for 30 to 48 h was transferred quickly (about 1 min total time) through three spots to wash it free of external label, then placed in a fourth spot to excrete $^{32}P$. After a time, usually 2 min, it was transferred to a fresh spot, and the contents of the previous spot were placed in a scintillation vial for counting. This transfer and collection of released $^{32}P$ was repeated several times, then the animal placed in a scintillation vial so that its remaining label could be counted. Washes were also counted to ensure that washing was sufficient.

When released material was collected for characterization by gel filtration, 100 μg P/L was added to inhibit microbial transformation. Sephadex G-25 gel was used in a 4 × 35 cm column. The gel separates molecules on the basis of molecu-

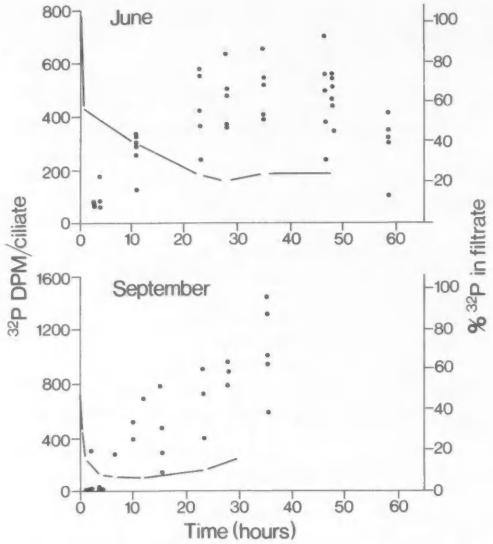


FIG. 1. The uptake of $^{32}$P label by whole plankton and by *S. viride* in lake water spiked with $^{32}PO_4$. The solid line shows the percent of $^{32}$P in the filtrate (0.2 μm). The dots indicate the amount of $^{32}$P in single *S. viride*. (1 DPM = 0.0166 Bq).

lar weight in the range of 0−5000. The sample was eluted with an aqueous solution of 0.3% sodium chloride and 0.02% sodium azide.

## Results

### THE UPTAKE OF $^{32}$P

In June, the amount of $^{32}$P taken up by *S. viride* incubated in lake water with $^{32}$P label reached a maximum by about 36 h (Fig. 1). There is a decline in the amount of $^{32}$P per ciliate after 48 h, perhaps because of gradual changes in the distribution of label among other plankton. In June, the amount of $^{32}$P label per *S. viride* was about 0.25% of the total activity per millilitre by 36 h, while in September it reached 0.7%. From the population estimates in Table 2, it can be estimated that *S. viride* contained about 0.7% and 0.1% of the total $^{32}$P in June and September, respectively.

To shed light on the mechanism by which $^{32}$P entered *S. viride*, a parallel time course experiment was run in September in which 10 μg P/L as phosphate was added to lake water in addition to the $^{32}$P label. As bacterial uptake is quickly saturated by such large nutrient additions (Lean 1981), more of the label enters the large (>12 μm) algae which, although less able to use low phosphate levels, are adept at rapid uptake and storage at high phosphate levels. The bacterial size fraction (0.2−1.0 μm) contains much less $^{32}$P, the 1- 5-μm fraction has about the same amount, while the large-sized fractions (5−12 μm, >12 μm) contain more, compared to unaltered lake water, when excess $PO_4$ is present (Fig. 2). The *S. viride* contain less $^{32}$P under the altered

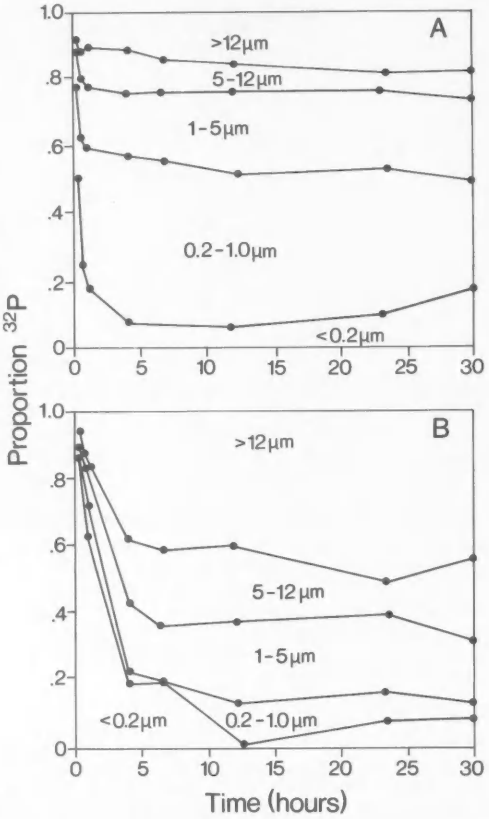


FIG. 2. The relative amount of $^{32}$P label in different size-classes of plankton with time in: (A) lake water; (B) lakewater plus added phosphate (10 μg P/L).

nutrient regime (Fig. 3). This indicates either that they feed largely on the 0.2- to 1.0-μm plankton fraction, and not upon the >5-μm fractions, or that they take up $^{32}$P from solution, and that the excess $PO_4$ acts as a competitive inhibitor.

Two experiments were conducted that bear directly on those two possibilities. Firstly, unlabeled *S. viride* were incubated in 0.2-μm filtered lake water containing 1.1 kBq/mL of carrier-free $^{32}PO_4$. Animals then were washed and counted at various times up to 65 min. No uptake of label was observed. Secondly, unlabeled *S. viride* were added to 72-h labeled lake water from the September experiments (Fig. 4). Their initial uptake of label was accelerated compared to the animals in the water when it was initially spiked with $^{32}PO_4$ (Fig. 1). These experiments confirm that $^{32}$P enters *S. viride* primarily via ingestion of labeled prey.

### EXCRETION EXPERIMENTS

Three phosphorus release experiments using *S. viride* are

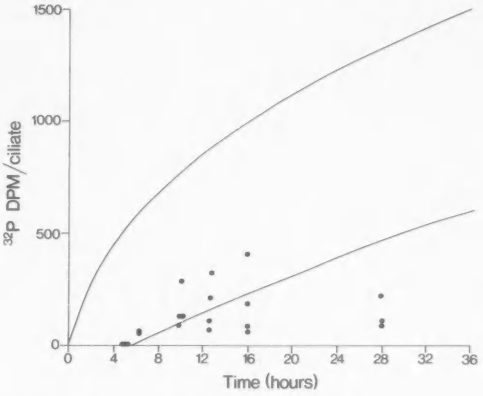


FIG. 3. The amount of $^{32}$P label in individual *S. viride* with time in lake water with added phosphate. The lines enclose the range of values observed in unaltered lake water (lower panel of Fig. 1).

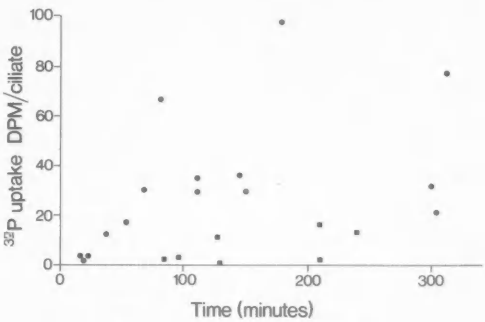


FIG. 4. The uptake of $^{32}$P label by individual *S. viride* when $^{32}PO_4$ is added to lake water (squares) and when unlabeled *S. viride* are added to labeled lake water (dots).

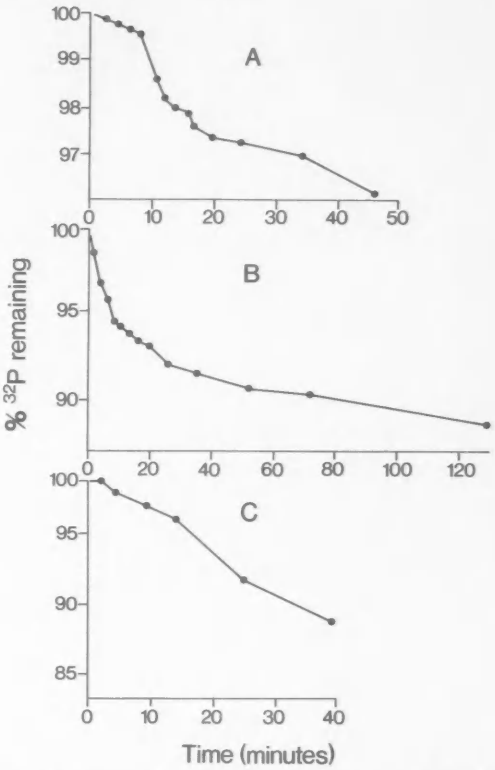


FIG. 5. Loss of $^{32}$P label by individual *S. viride* transferred to unlabeled lake water. Experiments A and B were conducted in June, and experiment C was conducted in September.

shown in Fig. 5. These records were chosen for their length and detail, but are typical of patterns and rates observed over a total of 10 experiments. In Fig. 5A, a slow, exponential loss of $^{32}$P was interrupted by a rapid loss phase, which later subsided. In Fig. 5B, rapid loss of $^{32}$P began immediately, and subsided after 20–60 min. In Fig. 5C, moderately rapid loss continued through the experiment. In all of these experiments, 89–96% of the label remained in the animals after 40 min. The most rapid loss rate observed (Fig. 5B, 1–8 min) corresponds to a turnover time of 2.7 h. Among the longer turnover times were 30 h (Fig. 5A, 1.25–10 min) and 66 h (Fig. 5B, 71–120 min).

Two experiments with *K. cochlearis* are shown in Fig. 6. In both there is a smooth, exponential decline interrupted by a precipitous decline. This decline probably is the loss of gut contents, but it is difficult to speculate as to whether it represents the normal pattern. The interval before the rapid decline may indicate a time when the animal is not feeding. In any event, a very large fraction of the total $^{32}$P loss occurs with this event. During the intervals before and after the rapid declines, excretion rates were very constant; the $r^2$ values for regressions of log (counts remaining) versus time were 0.998 ($n$ = 6, Fig 6A, 9–42 min), 0.996 ($n$ = 3, Fig 6A, 78–122 min), 0.998 ($n$ = 4, Fig 6B, 1–12 min), and 0.932 ($n$ = 5, Fig 6B, 32–50 min). This close fit to an exponential model not only reflects that the slow release of $^{32}$P was quite constant, but also that irregular excretion rates, as often occur with *S. viride* (Fig. 5), are not due to measurement error.

## CHARACTERIZATION OF THE RELEASED MATERIAL

The released material containing $^{32}$P was filtered through a 0.2-μm Nuclepore filter, then the filtrate was investigated using gel filtration. For *S. viride*, three animals were washed and allowed to lose label into a 1-mL bath containing an added 100 μg P/L as $PO_4$ for 15 min. During this time they lost about 1.5% of their $^{32}$P content. The released material appears to be largely phosphate (Fig. 7). The phosphate peak has a "shoulder" of material of low molecular weight (100–200),

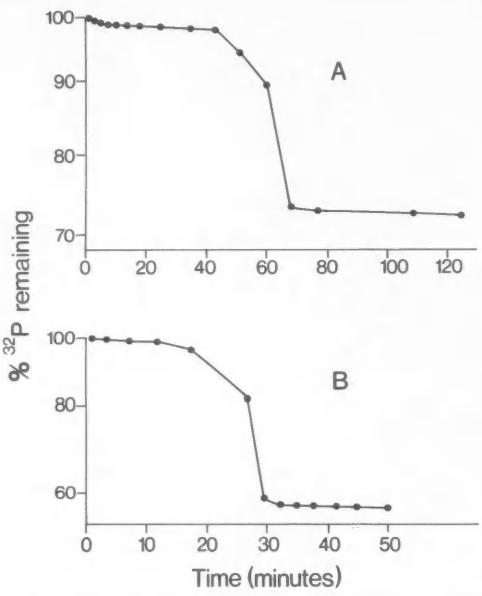


FIG. 6. Loss of $^{32}$P label by *Keratella cochlearis* transferred to unlabeled lake water.

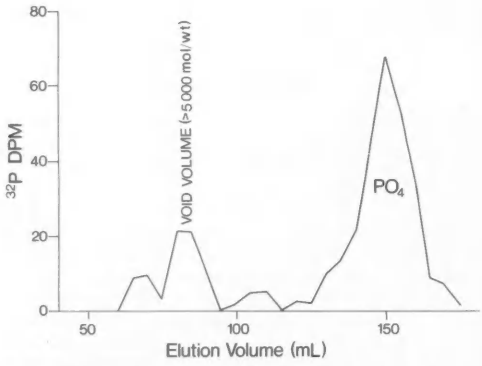


FIG. 7. Characterization of $^{32}$P label released by *S. viride* using Sephadex gel filtration.

similar in size to the XP fraction found in lake water (Lean 1973). In addition, there is a small amount of material of higher molecular weight (2000) and perhaps at void volume. Void volume is the volume of liquid contained by the column. Molecules eluted with this volume of eluant are too large to be impeded by the gel, i.e. >5000 mol wt. The activities of the eluted fractions often were very near background, so the minor peaks and irregularities in Fig. 7 and 8 probably should be ignored.

For *Keratella*, only one animal was used, and released

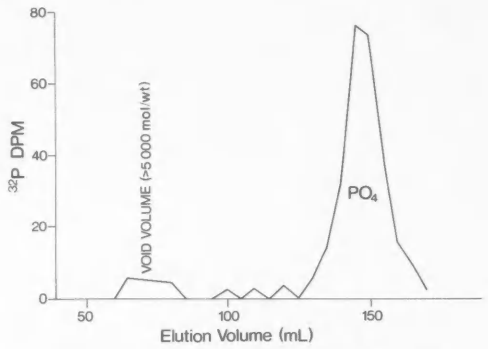


FIG. 8. Characterization of $^{32}$P label released by *K. cochlearis* using Sephadex gel filtration.

material was collected between 15 and 30 min after removal from labeled lake water. The animal lost about 3% of its initial amount of activity during this interval. The excreted material was almost entirely phosphate (Fig. 8). Neither *S. viride* nor *K. cochlearis* lost significant amounts of filterable (0.2 μm) material during these two experiments.

## Discussion

When $^{32}$P is added to lake water as phosphate, the ciliate *S. viride* gradually becomes labeled over a period of 30 h or more (Fig. 1). This is much longer than is required for the labeling of the plankton (Fig. 2 and 3), and is evidence that these animals do not rapidly exchange internal phosphorus with their environment. Other studies have shown that ciliates accumulated label linearly with time over the first few hours, but also report rapid turnover times. For example, Johannes (1964) reported linear uptake of $^{32}$P over 3 h, but a body equivalent excretion time (BEET) for *Euplotes vannus* of 20 min. Similarly, Buechler and Dillon (1974) report linear uptake over 3.5 h, but a BEET of 14–28 min for *Paramecium multimicronucleatum*. Not only is linear uptake over an interval longer than the BEET contradictory, but Buechler and Dillon (1974), who labeled their animals for only 1 h before performing their excretion experiments, must have greatly biased BEET values downwards with such an inappropriately short labeling time. Their animals would not have been uniformly labeled, as their calculation of BEET requires.

Our finding that *S. viride* acquires $^{32}$P primarily by ingesting labeled food rather than by direct uptake of phosphate is in agreement with the previous studies on ciliates (Johannes 1964; Buechler and Dillon 1974). This is an important point. If the reverse were true, these animals would have no net effect on phosphorus regeneration regardless of their release rate.

The method we used to examine phosphorus release, that is, measuring the release of label from a single animal over many successive intervals, is quite different to previous studies on both ciliates and crustacean zooplankton. Previous workers on ciliates incubated labeled animals in unlabeled medium, then used filtration to remove the animals from their

accumulated excretion. We adopted our method after discovering that we could not filter ciliates from lake water; we could not concentrate ciliates above a filter, and the $^{32}P$ from labeled animals went to the filtrate. We concluded that live *Strombidium* were too delicate to retain on a membrane filter. An advantage of our method is that it yields a record which sheds light on the release process. Obviously, these animals are not simply compartments exchanging with the environment; there appears to be at least two release processes (Fig. 5 and 6). Typically, there is a relatively rapid, discontinuous release of label from a relatively small compartment (although this compartment is much larger in *Keratella*, Fig. 6). Secondly, there is a relatively slow, probably continuous release from a second compartment. We hypothesize that the rapidly lost material is indigestable food vacuole or gut contents (defecation), while the slow process is the "excretion" measured in studies on crustacean zooplankton, where the animals are allowed to replace their gut contents with unlabeled food before release experiments begin. This would mean that the ciliate and the rotifer we studied have slow excretion rates comparable to crustacean zooplankton. It would also mean that direct comparison of BEETS, as obtained by Johannes (1964) and Buechler and Dillon (1974), and excretion of $^{32}P$, as measured by Peters and Rigler (1973), is inappropriate. The former include defecation, while the latter excludes it. If our interpretation of Fig. 5 and 6 is correct, phosphorus loss by defecation is probably vastly more important than by what we call excretion.

The bioavailability of defecated phosphorus is uncertain. For crustacean zooplankton, some of this material might be live algae (Porter 1973). Peters and Lean (1973) characterized the phosphorus material released (defecation + excretion) by *Daphnia rosea* feeding on yeast as about 70% filterable (0.45 μm). The filtrate fraction was about 47% phosphate (by retention on a hydrous zirconium oxide column) or 90% phosphate (by G-25 Sephadex filtration). The fraction not retained by the HZO column appeared to be labile, spontaneously releasing phosphate.

We did not find a particulate $^{32}P$ fraction among the material released by *Strombidium* or *Keratella* (10% of the total $^{32}P$ lost would have been enough to recognize). The released material was almost entirely phosphate (Fig. 7 and 8). We are led to believe that a significant portion of "defaecated" phosphorus might be available to algae and bacteria, and if so, the process of defecation might be much more important than the slow process we term excretion.

The earlier experiments of Johannes (1964) and Buechler and Dillon (1974) also suggest the existence of a small phosphorous pool with rapid turnover rate; in both studies, the uptake curve extrapolates back to a positive intercept. This would suggest that their uptake curves (and those in Fig. 1) represent assimilation or growth, rather than ingestion.

To understand the impact of changes in zooplankton communities, which might be caused directly by pollution, or indirectly by exploitation of fish populations or by additions of nutrients, it is necessary to verify or discard the concept that small zooplankton release more phosphorus per unit weight than large ones. Although this concept is very appealing because it is parallel to well-established negative relationships between weight-specific metabolism and body weight (e.g. Fenchel 1974; Gunther 1975; Allan 1976), it does not necessarily follow directly from them. For example, although the metabolic intensity of small zooplankters might be higher than large ones, they might feed more selectively and have higher assimilation efficiencies. Or they might have higher growth efficiencies and respire fewer of the organic phosphorus compounds they consume. To resolve these problems, we need to study all of the ways in which phosphorus is lost by zooplankters, quantify them, and then compare.


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# *Aeromonas salmonicida*: Relationship between Extracellular Growth Products and Isolate Virulence

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CIPRIANO, R. C., B. R. GRIFFIN, AND B. C. LIDGERDING. 1981. *Aeromonas salmonicida*: relationship between extracellular growth products and isolate virulence. Can. J. Fish. Aquat. Sci. 38: 1322–1326.

Extracellular growth products, extracted from culture supernatants of *Aeromonas salmonicida* by precipitation with ammonium sulfate and ethanol, were resolved into four fractions by ion-exchange chromatography. Although one of these (fraction II) possessed leukocytolytic activity, virulence of the pathogen was not associated with leukocytotoxicity. A positive correlation was established, however, between virulence and the toxicity of extracted material to cultured rainbow trout gonad (RTG-2) cells. The crude material and fractions II and III from virulent isolates were more toxic to RTG-2 cells than were similar preparations from avirulent isolates. Preparations from virulent isolates caused hemorrhaging, lesion development, and mortality when injected intramuscularly into brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*). Rainbow trout (*Salmo gairdneri*) were more resistant and lesions could be produced only with fraction II. Fraction II also possessed proteolytic activity.

*Key words*: *Aeromonas salmonicida*, furunculosis, pathogenicity, salmonids, extracellular products, cytotoxicity, leukocytolysis, proteolysis, fishes

CIPRIANO, R. C., B. R. GRIFFIN, AND B. C. LIDGERDING. 1981. *Aeromonas salmonicida*: relationship between extracellular growth products and isolate virulence. Can. J. Fish. Aquat. Sci. 38: 1322–1326.

La chromatographie sur échangeurs d'ions a permis de séparer en quatre fractions les produits de croissance extracellulaire extraits, par précipitation au sulfate d'ammonium et éthanol, du liquide surnageant de cultures de *Aeromonas salmonicida*. Bien que l'une de ces fractions (la fraction II) ait une activité leucocytolytique, la virulence de l'agent pathogène n'est pas liée à la leucocytotoxicité. Nous avons toutefois établi une corrélation positive entre la virulence et la toxicité, à l'endroit de cellules cultivées de gonade de truite arc-en-ciel (RTG-2), du matériel extrait. Le matériel brut et les fractions II et III extraites d'isolats virulents sont plus toxiques pour les cellules de RTG-2 que de semblables préparations provenant d'isolats non virulents. Les préparations d'isolats virulents causent des hémorragies, des lésions et des mortalités lorsque injectées par voie intramusculaire dans des ombles de fontaine (*Salvelinus fontinalis*) et des saumons atlantiques (*Salmo salar*). Les truites arc-en-ciel (*Salmo gairdneri*) sont plus résistantes, et, chez cette espèce, seule la fraction II cause des lésions. Cette fraction possède également une activité protéolytique.

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*AEROMONAS SALMONICIDA*, the etiologic agent of fish furunculosis, continues to be a major pathogen of cultured salmonids and the factors responsible for its virulence continue to be insufficiently understood. The pathology of the disease was extensively reviewed by McCraw (1952), Herman (1968), and McCarthy and Roberts (1980). Mortality is attributed to massive bacteremia that interferes with the host's blood supply and results in extensive tissue necrosis (Mackie et al. 1935). Mortality also has been related to hypoglycemic shock induced in the infected fish by the pathogen's use of the host's blood sugar (Field et al. 1944).

Other studies have indicated that the injection of extracellular material of *A. salmonicida* can reproduce the pathologic manifestations ascribed to the disease (Sakai 1977; Ellis et al. 1981). Leukopenic effects produced by injection of cell-free culture supernatants of this bacterium into rainbow trout (*Salmo gairdneri*) led Klontz et al. (1966) to suspect that a cytolytic component reduced leukocyte activity in infected fish. A leukocytolytic factor characterized as a glycoprotein

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was later isolated from culture supernatants and this component is believed to be partly responsible for the virulence of the bacterium (Fuller et al. 1977). Muscle degeneration and tissue necrosis, typical in fish with chronic furunculosis, are caused by an extracellular proteolytic enzyme (Shieh and MacLean 1975). Our research was undertaken in an effort to clarify the relation between virulence and toxic substances produced during growth of the bacterium.

## Materials and Methods

### Bacterial Cultures

*Aeromonas salmonicida* isolates (file stocks 3.10, 3.31, 3.95, and 3.101) were taken from the culture collection of the National Fish Health Research Laboratory (Leetown, WV); stocks of each isolate were stored in brain–heart infusion (BHI) broth at −80°C until used. Cultures 3.10 and 3.31 were avirulent at concentrations up to $10^6$ cells/mL when injected intraperitoneally into 3.8-g juvenile brook trout. Isolates 3.95 and 3.101, on the other hand, were highly virulent: the LD50 values were 138 and 38 cells per fish, respectively.

### Extracellular Growth Products

Crude growth materials were extracted from culture supernatants of each isolate by modifying the leukocytolytic factor extraction procedure used by Fuller et al. (1977). Isolates were thawed, seeded onto BHI agar slants, and incubated for 24 h at 20°C. Cultures incubated at 20°C for 24 h on a rotary shaker at 200 rpm were then inoculated into 2 L of aerated BHI broth and incubated for an additional 5 d at 20°C.

Cells were centrifuged from the broth culture at 3896*g* for 30 min at 4°C (the conditions under which we conducted all centrifugation in the present study). Extracts were prepared from the spent medium by precipitation with dry $(NH_4)_2SO_4$ crystals to give 80% saturation. The precipitate was sedimented by centrifugation, the supernatant was decant d, and the precipitate was redissolved in triple-distilled water. This material was dialyzed against several changes of distilled water for 18 h at 4°C, made to a 5% (w/v) sodium acetate concentration. Five volumes of 95% EtOH was then added to precipitate proteins, and the mixture was centrifuged. The supernatant was decanted and the precipitate was redissolved in triple-distilled water. This material was dialyzed against 0.01 mol/L $K_2HPO_4$ (pH 8.0) for 18 h at 4°C and adjusted to a standard volume of 30 mL with triple-distilled water.

Protein extracts were then fractionated by ion-exchange chromatography. DEAE Sephadex A-25 (20 g) was equilibrated to pH 8.0 in 0.01 mol/L $K_2HPO_4$ and packed in a 2 × 37 cm column, and 20 mL of the growth extract was applied and eluted from the column by a four-step salinity gradient from 0 to 1.2 mol/L.

Elution profiles were recorded at 280 nm with an ISCO type 6 optical unit with a UA-5 absorbance monitor (Instrument Specialty Company, Lincoln, NB). Fractions were identified as I–IV according to the order in which they were eluted from the column (Fig. 1). Proteins eluted at each step of the salt gradient were concentrated to 10.0 mL through a PM-10 ultrafilter (Amicon Corporation, Cambridge, MA) under

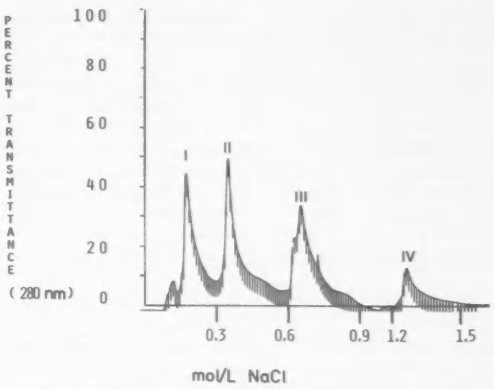


FIG. 1. Profile of crude extracellular *Aeromonas salmonicida* material from a Sephadex DEAE A-25 column. Fractions were eluted by a stepwise NaCl concentration gradient from 0.0 to 1.5 mol/L buffered in 0.01 mol/L $K_2HPO_4$ (pH 8).

nitrogen. Concentrates were dialyzed against 0.01 mol/L $K_2HPO_4$ for 18 h at 4°C. Protein determinations were made with Folin reagent (Lowry et al. 1951) with bovine serum albumin as a standard.

### Collection of Trout Leukocytes

Leukocytes were obtained from specific pathogen-free hatchery-reared brook trout (*Salvelinus fontinalis*) averaging 51 g. Fish were anesthetized in a solution of tricaine methanesulfonate in water (0.4 mg/mL) and caudal blood was drawn in to an equal volume of phosphate buffer containing heparin (100 units/mL). This suspension was layered over 19% Hypaque, prepared by diluting a 50% commercial Hypaque stock (Winthrop Laboratories, New York) with heparinized bufferin. After centrifugation at 150*g* for 15 min at 4°C, leukocytes at the plasma–Hypaque interface were removed with a pipette. Leukocytes were washed once in heparinized phosphate buffer, centrifuged for 5 min at 150*g* at 4°C, and resuspended in Eagle's minimal essential medium supplemented with fetal bovine serum (MEM-10). Cell densities were adjusted to 50% transmittance at 525 nm on a Spectronic-20 colorimeter (Bausch and Lomb, New York).

### Leukocytolysis Assays

Intact bacterial cells were incubated with trout leukocytes to estimate the leukocytolytic activity of each isolate. Bacterial suspensions of each isolate were standardized by adjustment with phosphate buffer to 95% transmittance at 525 nm. Bacterial numbers were estimated by dilution plate count (Miles and Misra 1938). Equal volumes (0.5-mL) of each bacterial dilution and leukocytes were mixed and incubated for 18 h at 20°C. Surviving leukocytes were estimated on the basis of trypan blue exclusion (Phillips 1973). A 50% reduction in leukocyte numbers was calculated by regression analysis.

The four fractions of the concentrated growth products from ion-exchange chromatography were then screened for leukocytolytic activity. This activity was found to be associated with fraction II (eluted at 0.3 mol/L NaCl). Ion-exchange fraction II from the virulent and avirulent isolates was then diluted in MEM-10, and 0.5 mL of each dilution was incubated with an equal volume of leukocytes for 18 h at 20°C. Assays were done in duplicate and results are expressed as the amount of protein required to cause a 50% reduction in leukocyte numbers (LC50).

### RTG-2 Cytotoxicity Assays

Cultured rainbow trout cells were used to study the generalized cytotoxicity of extracellular products of *A. salmonicida*. Confluent cell monolayers were prepared in flat-bottom microtiter plates and maintained in MEM-10 (Wolf and Quimby 1969). Cytotoxicity of the crude material and fractions I–IV from each isolate was measured by modifying existing tissue culture toxicity assays (Wolf and Quimby 1973). Medium was aspirated from test wells containing RTG-2 cells and replaced with 0.1 mL of the crude material or fractions diluted with MEM-10, and incubated for 24 h at 20°C. Control wells were aspirated and replaced with 0.1 mL of a 1:2 dilution of 0.01 mol/L $K_2HPO_4$ (pH 8.0) in MEM-10. After 24 h, plates were inverted, drained, rinsed with phosphate buffer, and fixed for 15 min in full-strength Formalin. The cells were then stained with 1% aqueous crystal violet for 5 min, rinsed with tap water, dried, and examined. The cytotoxicity of the respective bacterial fractions was determined by their ability to destroy cell monolayers, and a minimum cytotoxic level (the final dilution causing destruction) was determined for each extracellular preparation. Protein estimates were made as previously described.

### Injection of Growth Products

Juvenile Atlantic salmon (*Salmo salar*), brook trout, and rainbow trout, averaging 33, 51, and 66 g, respectively, were maintained in 64-L aquaria receiving 12.5°C flowing springwater at the rate of 2 L/min. The crude extract and fractions I, II, and III from one of the virulent isolates (3.101) were adjusted with phosphate buffer to 800 μg protein/mL. Because yields of fraction IV from chromatography were low, 200 μg/mL was the maximum protein concentration that could be developed for that sample.

Each of five Atlantic salmon, brook trout, and rainbow trout was injected intramuscularly with 0.1 mL of each growth product preparation. All fish were observed for 120 h after injection, mortalities were recorded, and carcasses were examined by culture of kidney material on BHI agar at 20°C for 48 h for evidence of bacterial infection.

Similar concentrations of the extracellular material were assayed for proteolytic activity with a Bio-Rad protease detection kit (Bio-Rad Laboratories, Richmond, CA).

Studies were also conducted to determine if individual extracellular components could act with a standard suspension of bacteria to enhance percent mortality. Suspensions of *A. salmonicida* (3.101) were prepared to contain one LD50 concentration of bacteria plus 5 μg protein (sublethal concentration) of the respective extracellular components per 0.1 mL. To prepare this suspension both the bacteria and the extracellular material were appropriately diluted in buffered saline.

Fingerling rainbow trout (averaging 7.1 g) were divided into groups of 20. Each fish in two groups was injected intraperitoneally with 0.1 mL of a bacterial–extracellular component suspension. Fish that were injected with 0.1 mL of the bacterium alone, the extracellular components alone, or buffered saline served as controls. All groups were observed for 14 d; dead fish were collected and confirmed for the presence of *A. salmonicida* in kidney tissue by direct FAT.

## Results

### Leukocytolysis Assays

Intact viable bacteria, crude growth media extracts, and chromatographic fraction II (Fig. 1) possessed leukocytolytic activity in brook trout (Table 1). The relative virulence of each isolate (as reflected by LD50 values in brook trout) was not related to either the direct action of bacteria when incubated with leukocytes or to the activities of partly purified fraction II from the growth media extracts. In fact, the most virulent isolate (3.101) possessed the least leukocytolytic activity. Consequently, the amount of extracellular materials extracted from cultures could not be correlated with isolate virulence (Table 2).

### RTG-2 Cytotoxicity Assays

Assays of crude growth media extracts and the chromatographic fractions indicated that toxicity to RTG-2 cells was related directly to virulence (Table 3). The relation existed for both the crude material and for fraction II. The apparent correlations between virulence and cytotoxicity of fractions I, III, and IV were not real ($t$-test, $P < 0.05$).

Table 1. Analysis of leukocytolysis assay determining the number of bacteria and micrograms of protein chromatographic fraction II required to cause 50% reduction of leukocytes in brook trout.

| Isolate stock no. | LD50 values for brook trout | No. of bacteria producing 50% leukocyte reduction | Amount of protein (μg) (fraction II) producing 50% leukocyte reduction |
|---|---|---|---|
| 3.101 | 38 | $5.4\times10^6$ | 105 |
| 3.95 | 138 | $1.1\times10^4$ | 19 |
| 3.31 | $>10^6$ | $9.7\times10^4$ | 16 |
| 3.10 | $>10^6$ | $9.4\times10^5$ | 24 |

TABLE 2. Total protein estimates of the extracellular components obtained from isolates of *Aeromonas salmonicida*.

| Isolate | Extracellular component (μg protein/mL) | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Avirulent (3.10) | 1090 | 822 | 1209 | 87 |
| Avirulent (3.31) | 698 | 921 | 448 | 64 |
| Virulent (3.95) | 202 | 844 | 1367 | 48 |
| Virulent (3.101) | 409 | 485 | 409 | 35 |

TABLE 3. Minimum protein concentrations of crude and partly purified extracellular fractions of *Aeromonas salmonicida* required to produce complete destruction of cultured trout cells.

| Isolate stock no. | Protein (μg) required to kill RTG-2 cells | | | | |
|---|---|---|---|---|---|
| | Crude material | Fractions | | | |
| | | I | II | III | IV |
| 3.101 | 324 | 35 | 14 | 37 | 10 |
| 3.95 | 691 | 71 | 24 | 35 | 11 |
| 3.31 | 1708 | 28 | 214 | 52 | 16 |
| 3.10 | 1823 | 74 | 177 | 100 | 13 |

TABLE 4. Number of fish that died within 24 h after intramuscular injection of crude and partly purified extracellular products of a virulent (3.101) *Aeromonas salmonicida* isolate into five fish of each of three species.

| Species | Crude | Extracellular fraction | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| Atlantic salmon | 2 | 3 | 4 | 1 | 0 |
| Brook trout | 2 | 2 | 4 | 0 | 0 |
| Rainbow trout | 0 | 0 | 0 | 0 | 0 |

### IN VIVO TOXICITY STUDIES

Injection of the chromatographic fractions from a virulent (3.101) isolate indicated that at least three of these components were toxic to fishes and that their activities differed among resistant and susceptible species. Mortality occurred in Atlantic salmon and brook trout injected with the crude growth extract fraction I or II (Table 4). The injection of these components was usually accompanied, within 48 h, by hemorrhage at the vent and fins and inflammation at the site of injection. Although only one Atlantic salmon died from injection of fraction III, the survivors and all the brook trout similarly injected hemorrhaged at the base of fins, site of injection, and the mouth. Fraction III produced extensive petechia throughout internal organs.

By contrast, neither gross pathology nor mortality was observed when rainbow trout were injected with the crude material or fraction I or III. However, all the rainbow trout injected with fraction II developed characteristic furunclelike lesions at the site of inoculation within 120 h. Inflammation at the site of injection was extensive within 24–48 h, and became progressively hemorrhagic after 72 h; by 96 h these areas were necrotic, devoid of scales, and hemorrhagic at the periphery of the lesions; by 120 h the lesions ruptured, resulting in the development of "furuncules" typically associated with the disease.

No mortality and only slight inflammation at the site of injection were noted among fishes injected with fraction IV, perhaps because of the much lower concentration at which this component was injected.

Both the crude growth material and fraction II were active in the protease assay, whereas fractions I, III, and IV were not.

Mortality was enhanced by injecting rainbow trout with sublethal concentrations of the individual chromatographic fractions plus a standard LD50 concentration (9300 cells per fish) of the bacterium. No mortalities were observed among control groups that were injected either with buffered saline or 5 μg/mL of the respective extracellular component. Kidney tissue that was examined from each of these fish 14 d after injection was negative for *A. salmonicida* by direct FAT.

Two groups of trout injected with the bacterium alone had a 45% mean mortality (9 of 20 individuals per group). Those injected with bacteria plus fraction I had a 73% mean mortality (16 and 13 deaths per group), those injected with bacteria plus fraction II had an 88% mean mortality (18 and 17 deaths per group), those injected with bacteria plus fraction III had a 90% mean mortality (20 and 16 deaths per group), and those injected with bacteria plus fraction IV had a 73% mean mortality (14 and 15 deaths per group). *Aeromonas salmonicida* was confirmed in kidney tissue of all fish that had been injected with bacteria.

## Discussion

Our results suggest that leukocytolytic and proteolytic activities of *A. salmonicida* are dual expressions of a single component, chromatographic fraction II. However, additional evidence is necessary to confirm this point. Fractions I, III, and IV possess cytolytic activities to RTG-2 cells that are unrelated to the bacterium's proteolytic and leukocytolytic activities. It is not know whether these activities are caused by different toxic substances or different forms of the same substance.

Fuller et al. (1977) suggested that the virulence of *A. salmonicida* was reflected by the lytic effects of its growth products on trout leukocytes. The present work indicated that this conclusion was drawn from an unfortunate choice of a virulent and an avirulent isolate. The concentration of leukocytolytic substances produced by a given isolate now seems to be variable and unrelated to virulence. More closely related to virulence is the generalized cytotoxicity of the bacterial growth products as measured by our RTG-2 cytotoxicity assays.

On the basis of results with crude extracellular growth product preparations, Sakai (1977) indicated that a protease is the most pathogenic element produced by *A. salmonicida*, an indication that our results tend to support. Furthermore, the effects ascribed to proteolytic activity are similar to those of chromatographic fraction II. Although each of the extra-

cellular components injected in combination with a single LD50 dose of the bacterium enhanced mortality, fraction II, when injected alone, was the only component that was active in rainbow trout. The diminished response of rainbow trout to injection with other fractions that induce pathology in both Atlantic salmon and brook trout may partly account for the greater resistance of rainbow trout to furunculosis. Additional studies are needed to clarify this point and to investigate the immunogenic potential of each extracellular component.

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# Prediction of Egg Development and Mortality Rates in Capelin (*Mallotus villosus*) from Meteorological, Hydrographic, and Biological Factors[1]

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FRANK, K. T., AND W. C. LEGGETT. 1981. Prediction of egg development and mortality rates in capelin (*Mallotus villosus*) from meteorological, hydrographic, and biological factors. Can. J. Fish. Aquat. Sci. 38: 1327–1338.

We studied the factors governing in situ egg development and mortality rates in beach spawning capelin (*Mallotus villosus*). Time to hatching was strongly influenced by the average incubation temperature which varied between years and between cohorts in a single year, and between positions on the beach (high, mid, low tide zone). Average incubation temperatures were determined by a complex of variables which included water temperature, maximum and minimum air temperature, and hours of sunlight. Mortality rates were both spatially and temporally variable. Over 70% of the temporal and spatial variance in egg mortality was attributed to the interacting effects of egg density, water temperature, maximum and minimum air temperature, hours of sunlight, rainfall, and tidal amplitude. Multivariate equations for predicting egg mortality as a function of these variables are given. The principal climatic and hydrographic variables influencing egg mortality vary only slightly over a 7° range of latitude encompassing the main spawning sites of the eastern Newfoundland and southern Labrador coasts. The potential thus exists for synchronous year to year changes in egg mortality over this spawning range as a result of annual variations in climate and hydrographic conditions. Such synchronous changes may contribute to the substantial year to year fluctuations in capelin year-class strength.

*Key words*: capelin, *Mallotus villosus*; development, mortality, meteorology, hydrology, temperature, intertidal zone, year-class, egg, beach spawning

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Nous avons examiné les facteurs influençant le développement et les taux de mortalités in situ d'œufs de capelan (*Mallotus villosus*) frayant sur les plages. La duré d'incubation est fortement influencée par la température moyenne d'incubation qui varie d'une année à l'autre et d'une cohorte à l'autre d'une même année, et aussi selon l'endroit de la plage (zone de marée haute, moyenne ou basse). Les températures moyennes d'incubation sont déterminées par un ensemble de variables, dont la température de l'eau, la température maximale et minimale de l'air et les heures d'ensoleillement. Les taux de mortalité varient tant dans l'espace que dans le temps. Plus de 70% de la variance temporelle et spatiale de la mortalité des œufs, température de l'eau, température maximale et minimale de l'air, heures d'ensoleillement, précipitation et marnage. Nous donnons des équations multidimensionnelles permettant de prédire la mortalité des œufs en fonction de ces variables. Les principales variables climatiques et hydrographiques qui influent sur la mortalité des œufs ne varient que légèrement sur une distance latitudinale de 7°, comprenant les principaux lieux de ponte des côtes est de Terre-Neuve et sud du Labrador. Il est donc possible qu'il y ait synchronisme d'une année à l'autre dans les variations de mortalité des œufs sur ces lieux de ponte, résultant des variations annuelles des conditions climatiques et hydrographiques. De tels changements synchrones peuvent contribuer aux importantes fluctuations annuelles d'abondance des classes d'âge du capelan.

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NUMEROUS marine fishes utilize shallow water (coastal or shoal) and intertidal regions for spawning. Demersal spawners utilizing such areas typically select spawning microhabitats primarily on the basis of substrate or vegetation type, e.g. surf smelt, *Hypomesus pretiosus* (Schaefer 1936; Loosanoff 1937); Pacific herring, *Clupea pallasii* (Taylor 1964); Norwegian herring, *Clupea harengus* (Runnstrom 1941); White Sea herring, *Clupea harengus maris-albi* (Soin 1971); and capelin, *Mallotus villosus* (Templeman 1948; Saetre and Gjøsaeter 1975). Strict spawning site specificity frequently results in dense concentrations of eggs, a situation not normally experienced by the eggs of pelagic spawners. Numerous biotic and abiotic variables have been identified as potential regulators of egg development rate and survival in these demersal spawners. Several authors have reported a strong negative effect of density on survival principally as a result of limitation of oxygen exchange and excess accumulation of excretory products (Lea 1930; Runnstrom 1941; Wickett 1954; Hempel 1963; Bowers 1969; Galkina 1971; Taylor 1971; Bailey et al. 1980). Substrate characteristics, desiccation and stranding, and temperature extremes have also been shown to influence the survival of demersal eggs (Galkina 1971; Soin 1971; Jones 1972; Blaxter 1956; Alderdice and Velsen 1971). The principal factor controlling egg development rate is temperature, there being a pronounced inverse relationship between incubation temperature and time to hatching (Gray 1928; Brett 1956; Alderdice and Forrester 1971; Ferraro 1980; Coombs and Hiby 1979). The relative importance of biotic and abiotic factors as regulators of egg development rates and mortality for any given species is poorly known and predictive models describing the interaction of these variables and their effects on egg development and mortality rates are virtually nonexistent. This situation has arisen partly because many egg surveys have been conducted primarily to assess the size of the adult spawning stock without reference to agents of mortality (e.g. those studies by Saville 1964; Gjøsaeter and Saetre 1974; Tanaka 1974) and partly because of the logistic and technical difficulties involved in systematic studies on egg development and survival in demersal spawning beds. The result has been a general lack of dependable quantitative estimates on the magnitude of egg mortality for most marine fishes (see reviews by Hempel 1963; Dahlberg 1979).

The capelin, a dominant element of the food web of the North Atlantic (Bailey et al. 1977), exhibits a beach spawning behavior (Sleggs 1933; Templeman 1948) which obviates these logistic difficulties and makes detailed in situ investigations of biotic and abiotic regulators of egg development and survival a practical possibility. Beach spawned eggs adhere strongly to the intertidal beach sediments and are buried over the breadth of the intertidal zone by wave and tidal action. Eggs hatch after a 2- to 3-wk period of development and the larvae are subsequently flushed from the sediments by wave action induced by onshore winds (Frank and Leggett 1981). These features of the egg incubation period make possible systematic quantitative studies of factors influencing egg development and mortality.

In this paper we report the results of a study designed to (1) quantify the effect of a series of variables on the physical and biological characteristics of the egg incubation site and (2) develop predictive models relating these effects to observed spatial and temporal trends in egg development and mortality rates.

## Study Location

The study was conducted at Bryant's Cove, Conception Bay, Newfoundland (47°40′36″N, 53°11′24″W) during the spring and summer of 1978 and 1979. The capelin spawning beach at Bryant's Cove is typical of spawning beaches throughout eastern Newfoundland and Labrador (Sleggs 1933; Templeman 1948). A detailed description of the site is given in Frank and Leggett (1981).

## Sampling Methods

In each of the 2 yr of our study two separate spawnings occurred in each season permitting the independent study of two egg cohorts in each year. The spawning times were June 14–20 and July 5–9, 1978, and June 4–8 and June 21, 1979. We sampled eggs by coring the beach twice weekly beginning immediately after the commencement of spawning and continuing until all eggs had hatched and all larvae had exited the beach. Cores were made at three sampling sites in 1978 and two sites in 1979. At each site one core was drawn from each of the high tide (HT), mid tide (MT), and low tide (LT) regions of the intertidal zone. All sampling was conducted during daylight hours and at the time of low tide. To obtain each core we forced the 5.5-cm internal diameter steel corer vertically into the sediments to the maximum depth possible. The surrounding substratum was then removed and a spatula was slipped under the corer sealing its opening to retain the sample. We ensured that no eggs occurred in the sediments at depths greater than the maximum penetration of the corer. A calibrated plunger was used to determine the total depth of the core and to remove the sample from the corer in discrete 2-cm slices. Sectioned core samples were preserved in 4% buffered Formalin.

## Egg Density and Distribution

All eggs were counted from each 2-cm interval of the 1978 core samples. Egg densities were expressed as geometric means of the replicate samples. Analysis of these data revealed no depth-related trends in distribution or mortality (see below); consequently only the 4- to 6-cm interval was examined in 1979.

Average egg densities in the beach gravel immediately after spawning ranged from 90 to 140 eggs/cm$^3$. Egg densities were slightly, but not significantly, higher in 1979 (Table 1). Eggs were not uniformly distributed on the beach. In general egg densities immediately following spawning were greatest at the high tide location, but subsequently the numbers of eggs there declined more rapidly with time because median time to hatching increased from the high to low tide positions (see below) (Table 1). The maximum depth of egg deposition was near uniform over the beach, averaging 10–12 cm. No significant vertical zonation was found (Table 2).

## Development Rates

In situ egg development rates were determined in two ways. In 1978 we followed the development of eggs sampled

TABLE 1. Spatial distribution and temporal changes in capelin egg density (geometric mean no./cm$^3$) and percentage dead eggs in four egg cohorts. Egg age was taken as the time since the midpoint of each period of active spawning.

| | | | | Location | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | HT | | MT | | LT | |
| Year | Cohort | Day | Age | Density | Mortality | Density | Mortality | Density | Mortality |
| 1978 | I | June 21 | 4 | 62.7 | 48.0 | 46.9 | 34.4 | 11.9 | 14.8 |
| | | June 24 | 7 | 92.6 | 50.2 | 46.4 | 41.8 | 34.1 | 34.2 |
| | | June 27 | 10 | 27.6 | 42.4 | 35.4 | 53.5 | 53.7 | 59.7 |
| | | June 30 | 13 | 11.6 | 38.8 | 46.0 | 37.4 | 18.5 | 32.7 |
| | | July 4 | 17 | 1.4 | 34.1 | 3.2 | 23.5 | 13.0 | 15.0 |
| | | July 8 | 21 | 2.6 | 12.8 | 2.2 | 11.8 | 2.0 | 13.9 |
| | II | July 12 | 5 | 35.3 | 22.3 | 48.2 | 23.1 | 86.9 | 24.2 |
| | | July 15 | 8 | 53.6 | 30.3 | 40.8 | 20.6 | 88.4 | 13.8 |
| | | July 19 | 12 | 52.0 | 30.5 | 42.2 | 24.1 | 35.4 | 23.5 |
| | | July 22 | 15 | 17.2 | 13.6 | 18.4 | 14.0 | 31.1 | 20.5 |
| | | July 26 | 19 | 5.7 | 21.6 | 7.7 | 23.5 | 7.4 | 21.2 |
| | | July 29 | 22 | 0.8 | 8.3 | 2.2 | 12.3 | 5.8 | 14.5 |
| 1979 | I | June 9 | 1 | 78.6 | 28.1 | 29.5 | 9.3 | 26.5 | 9.3 |
| | | June 13 | 5 | 124.9 | 27.3 | 57.1 | 35.7 | 116.2 | 20.3 |
| | | June 16 | 8 | 74.1 | 90.1 | 46.7 | 38.3 | 53.7 | 31.8 |
| | | June 20 | 12 | 25.0 | 41.1 | 24.2 | 27.4 | 3.9 | 28.1 |
| | | June 22 | 14 | 30.6 | 34.1 | 84.7 | 20.4 | 66.3 | 11.9 |
| | II | June 27 | 6 | 71.7 | 42.0 | 32.9 | 44.3 | 90.7 | 23.7 |
| | | June 30 | 9 | 138.6 | 61.4 | 48.3 | 51.6 | 69.8 | 38.9 |
| | | July 4 | 13 | 46.8 | 41.3 | 17.2 | 32.2 | 49.2 | 26.7 |
| | | July 7 | 16 | 22.7 | 16.2 | 20.3 | 43.0 | 32.4 | 27.0 |
| | | July 11 | 20 | — | — | 23.3 | 27.8 | 14.7 | 30.6 |
| | | July 14 | 23 | 4.2 | 23.1 | 11.3 | 6.6 | 9.4 | 11.9 |

TABLE 2. Correlation coefficients between depth of egg depostion, percentage egg mortality, and egg density (no./cm$^3$) for 1978. Egg mortality and density were calculated for each 2-cm depth strata. Two egg cohorts were combined for the analysis. *$P > 0.05$.

| Intertidal location | Depth range (cm) | % egg mortality | Egg density | $n$ |
|---|---|---|---|---|
| HT | 0–14 | −0.480 ns* | 0.151 ns | 13 |
| MT | 0–12 | −0.343 ns | −0.446 ns | 11 |
| LT | 0–12 | −0.194 ns | −0.523 ns | 12 |

from the beach by coring. In 1979 egg development rates were determined using experimental chambers. Open-ended cylindrical Plexiglas chambers (5 cm diameter × 30 cm long) perforated with hundreds of 10-mm-diameter holes which were covered with 0.15-mm Nitex were filled with egg-bearing gravel collected immediately after spawning; the ends were covered with 0.15-mm Nitex. The chambers were buried in locations adjacent to three temperature probes located in the intertidal zone. A fourth chamber was located directly below the low tide level (subtidal). Egg samples were removed from each cylinder daily and preserved in 4% Formalin. The duration of daily sampling ranged from 10 to 19 d. Two separate experiments of this type were run. The first experiment was carried out from June 9 to June 21 and the second from June 23 to July 7. To determine the degree of egg development we soaked the preserved core and chamber samples for a minimum of 4 h in Stockard's solution which turns the embryo white and leaves the yolk clear (Bonnet 1939). At least 50 eggs from each sample were examined and their stage of development noted using an 8-point scale based on a modification of Fridgeirsson's (1976) description of capelin egg development:

| Stage index | Characteristics |
|---|---|
| 1 | Encompasses formation and cleavage of blastodisk; ends with formation of blastula |
| 2 | Formation of basic embryonic tissue layers—gastrulation |
| 3 | Pronounced thickening of tissue in head and tail regions due to formation of rudimentary organs and symmetric forebrain bulges; optic bulb and crack visible on the bulge of forebrain; somites developing |
| 4 | Full development of main organs; hindgut of embryonic body developing; yolk sac appears partially divided; eyes lightly pigmented and visible |
| 5 | Darkening of eye pigmentation; hindgut, anus, and somite development complete; appearance of hatching cells |
| 6 | First separation of head from yolk sac; single row of black pigment cells (chromatophores) visible along ventral midline |

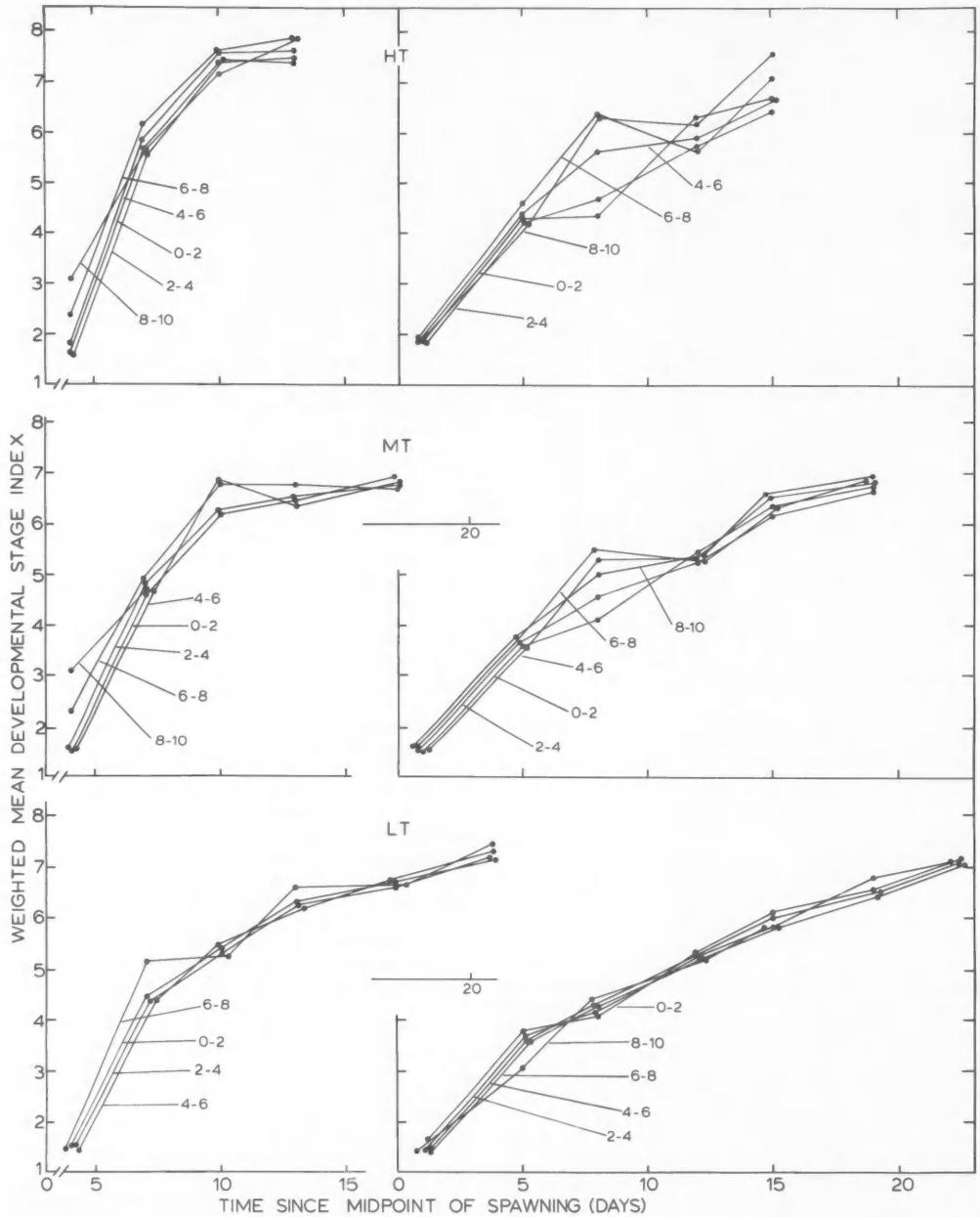


FIG. 1. In situ egg development rate at successive 2-cm depth strata at high (HT), mid (MT), and low (LT) tidal locations in 1978.

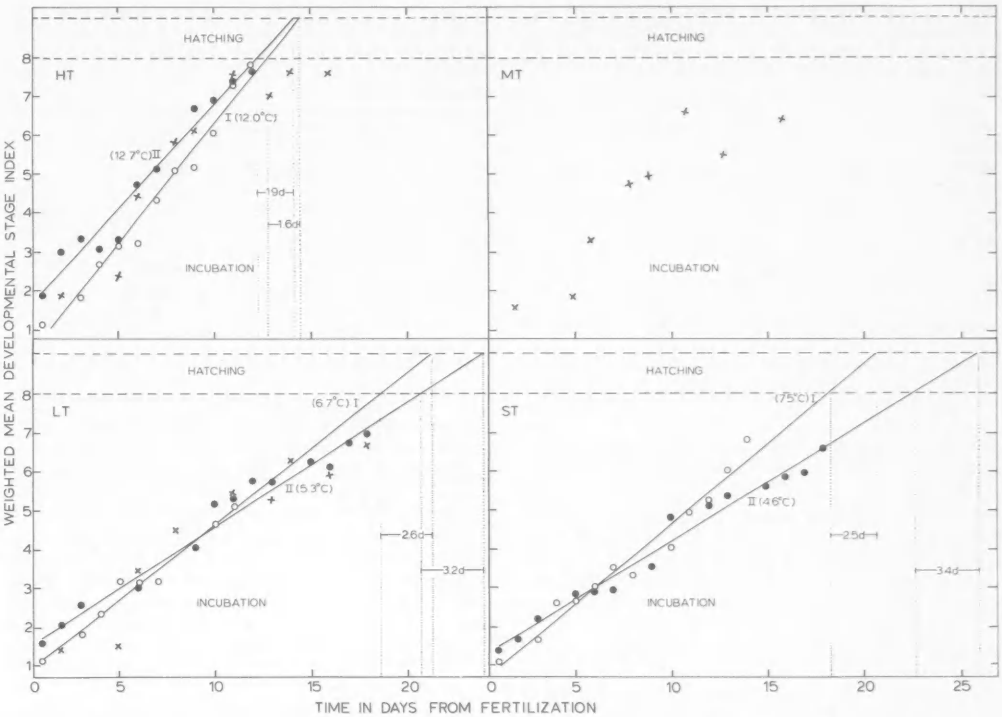


FIG. 2. Egg development rate as determined from cannister experiments conducted at high (HT), low (LT), and subtidal (ST) locations (experimental data lacking for mid tide (MT)). Two experiments, I (○) and II (●), were conducted at each location. Average incubation temperature during each experiment indicated as I (12.0°C), etc. Dotted vertical lines indicate duration of the hatching interval. ×s indicate in situ estimates of egg development rates as determined from core samples.

7 More intense pigmentation of eyes and chromatophores prior to hatching; distinct lifting of head from yolk; head pressing against chorion

8 Hatching, full yolk sac; mean length at hatching = 4.9 mm

A central measure of egg development for a given sample was obtained by the following equation:

$$\bar{s} = \sum_{i=1}^{8} s_i e \Big/ \sum_{i=1}^{8} e_i$$

where $\bar{s}$ is the weighted mean developmental stage index, $s_i$ is the developmental stage index obtained from the preceding table, and $e_i$ is the number of eggs designated as falling into a particular developmental stage.

We observed no consistent depth-related trends in egg development rates (Fig. 1). Development rates and median time to hatching did, however, vary with beach location. This pattern was evident in both the field and experimental determinations of development rate (Fig. 2). Development rates were fastest at high tide and declined stepwise to low tide. Low tide and subtidal rates did not differ. Median time to hatching increased from high to low tide, averaging 12−13 d at high tide and 18−19 d at low tide in the core samples and 13 d at high tide and 22 d at low tide in the experimental chambers.

## Mortality

Dead eggs were distinguished from live ones by their opaqueness, the perivitelline space being distinctly absent in dead eggs (Southward and Demir 1974). Egg mortality within each 2-cm interval or over the entire core was expressed as a weighted percentage of the replicate samples and was calculated by the formula:

$$\text{egg mortality} \times 100 = \left(\sum_{i=1}^{k} d_i\right) \Big/ \sum_{i=1}^{k} e_i$$

where $e_i$ is the total number of eggs in the $i$th sample, $d_i$ is the

TABLE 3. Three-factor analysis of variance and a posteriori means test on the replicate whole core estimates of egg mortality. The factors were year (1978, 1979), intertidal location (HT, MT, LT), and egg age. (>) indicates significant differences at $P < 0.05$.

| Source | df | P |
|---|---|---|
| Year (Y) | 1 | <0.05 |
| Locale (L) | 2 | <0.001 |
| Age (A) | 18 | <0.001 |
| Y × L | 2 | <0.05 |
| Y × A | 3 | <0.001 |
| L × A | 35 | <0.05 |
| Y × L × A | 6 | ns |
| Error | 103 | |
| Total | 170 | |

Duncan's multiple-range test
Level of alpha = 0.05, df = 103

*Between years*

| Year | 1979 | > 1978 |
|---|---|---|
| Mean | 30.4% | 26.2% |
| *n* | 64 | 107 |

*Between locations*

| Location | HT | > MT | > LT |
|---|---|---|---|
| Mean | 30.4% | 27.2% | 23.2% |
| *n* | 55 | 58 | 58 |

total number of dead eggs in the *i*th sample, and *k* is the number of samples. This method of analysis assumed that dead eggs did not accumulate in the beach gravel and hence the number and/or proportion of dead eggs in consecutive samples was not additive. No evidence of accumulation was found (Table 1). Examination of the egg collections from both years revealed the presence of substantial numbers of maggots of the beach fly *Fucellaria*, their densities ranging from 5 to 40 maggots/cm$^3$ in egg samples drawn primarily from the high tide location. Maggot density and capelin egg density were unrelated ($r = 0.153$, $P > 0.05$). Templeman (1948) suggested that these maggots are major predators of dead eggs. This may, in part, explain the absence of an accumulation of dead eggs in the gravel. A preferred method of analyzing egg mortality, based on instantaneous daily mortality rates calculated from the number of live eggs in consecutive samples, was not possible because the relative contributions of hatching and mortality to reductions in egg numbers over time could not be separated.

Egg mortality and depth of deposition were not significantly correlated (Table 2). The absence of depth-related development and mortality differs strikingly from the inverse relationship between development and mortality and depth of deposition reported for demersal spawning herring (Runnstrom 1941; Parrish et al. 1959; Blaxter 1956; Bowers 1969; Baxter 1971).

Because no depth-related trends in development rate or mortality were evident we based our subsequent analysis of factors influencing these variables on whole core estimates. A three-way ANOVA incorporating year, intertidal location (high, mid, low tide), and egg age (time since median spawning) revealed significant differences in mortality between years, between locations, and between egg ages. Mortality was significantly higher in 1979 and declined from high to low tide (Table 3).

TABLE 4. Two-way analysis of variance and a posteriori means test on the replicate whole core estimates of egg mortality for (A) 1978 and (B) 1979. In (A) combinations of underlined means do not differ significantly at $P < 0.05$ and (B) (>) indicates significant differences at $P < 0.05$.

(A) 1978

| Source | df | P |
|---|---|---|
| Locale (L) | 2 | <0.05 |
| Age (A) | 11 | <0.001 |
| L × A | 22 | ns |
| Error | 71 | |
| Total | 106 | |

Duncan's multiple-range test
Level of alpha = 0.05, df = 71

*Between locations*

| Location | HT | MT | LT |
|---|---|---|---|
| Mean | 29.8% | 25.8% | 23.3% |
| *n* | 35 | 36 | 36 |

(B) 1979

| Source | df | P |
|---|---|---|
| Locale (L) | 2 | <0.001 |
| Age (A) | 10 | <0.001 |
| L × A | 19 | <0.01 |
| Error | 32 | |
| Total | 63 | |

Duncan's multiple-range test
Level of alpha = 0.05, df = 32

*Between locations*

| Location | HT | > MT | > LT |
|---|---|---|---|
| Mean | 40.5% | 29.4% | 22.9% |
| *n* | 20 | 22 | 22 |

Second-order interactions (Table 3) dictated that the effects of age and location be evaluated within years. A two-way ANOVA (Table 4) revealed that within years (1) mortality declined from high to low tide, (2) mortality differed significantly with age, and (3) the level of this age-related mortality was site specific.

We also assessed the possible impact of beach maggots on egg survival. A predetermined number of live eggs and maggots were combined in open-ended cylindrical Plexiglas chambers (5 cm diameter × 6 cm long) identical in design to those used in the egg development experiments. A range of egg to maggot ratios approximating their densities in the beach gravel were tested against control chambers lacking maggots. A total of four experiments, each lasting 4 d and employing 15 chambers, were conducted in situ at the high tide location. No effect of maggots was found. Control chambers frequently exhibited egg mortalities similar to or greater

TABLE 5. Summary of parameters obtained from stepwise regression of mean daily gravel temperature at each of three intertidal locations against the daily means of the measured physical variables. Order of addition of variables in the stepwise regression is shown.

| Intertidal location | Independent variables: Water temp., °C | Max. air temp., °C | Bright sun, h | Min. air temp., °C | Intercept | $r^2$ | $P$ |
|---|---|---|---|---|---|---|---|
| High | 0.60716 | 0.14174 | 0.34755 | 0.39216 | −0.29690 | 0.76 | <0.001 |
| Order | 1 | 4 | 2 | 3 | | | |
| Mid | 0.50593 | — | 0.16193 | 0.19021 | 2.42593 | 0.76 | <0.001 |
| Order | 1 | | 2 | 3 | | | |
| Low | 0.81150 | — | — | — | 1.71160 | 0.80 | <0.001 |
| Order | 1 | | | | | | |

than those containing eggs and maggots. These findings support Templeman's (1948) belief that the principal food of maggots is dead eggs.

Spatial differences in egg mortality have been reported for other intertidal spawners. Soin (1971) demonstrated that eggs of the White Sea herring incubating in the intertidal zone experienced 100, 70, and 30% mortality at the high, mid, and low tide intertidal locations, respectively. Tester and Stevenson (1949) reported that survival of Pacific herring eggs at the 0-tide level was greater than at regions above or below this level. These data are at odds with those of Jones (1972) who subjected eggs of the Pacific herring to laboratory-simulated intertidal conditions, varying the ratio of exposure to submergence times. Unexposed eggs experienced mortality rates of 13% as compared with 31% at 8 h exposure twice daily.

## Meteorological, Hydrographic, and Biological Regulators of Development Rate and Survival

We hypothesized that the spatial and temporal differences in development and mortality rates described above reflect the action of a complex of biotic and abiotic variables. To study the importance of abiotic factors we first quantified the influence of a series of variables which we believed, a priori, would be potentially important regulators of egg development rate and mortality. The variables and our justification for selecting them are as follows.

1) Water temperature, maximum and minimum air temperatures, and daily hours of sunlight. An extensive literature (see for example Bonnett 1939; Brett 1956; Alderdice and Forrester 1971; Blaxter 1956; Taylor 1971; Dahlberg 1979) links incubation temperatures directly to egg development and mortality rates. Air and water temperatures and solar heating can be expected to influence incubation temperatures in beach spawning species such as capelin.

2) Tidal amplitude. Both the frequency of inundation and the relative importance of water and atmospheric temperatures and solar heating as determinants of incubation temperatures were expected to differ between high, mid, and low tide and to vary temporally with tidal amplitude. The frequency and extent of tidal flushing may also influence the extent of accumulation of metabolic wastes in the gravel at various locations on the beach (Penaz 1965; Burkhalter and Kaya 1977; Bailey at al. 1980).

3) Daily rainfall. We hypothesized that rainfall could influence the salinity of the rearing environment thereby influencing survival. Furthermore, depending on porosity and other variables, this effect could vary from low to high tide locations.

Meteorological and hydrographic data relating to the study periods were obtained from various sources. Hourly wind direction and velocity summaries were obtained from the Holyrood meteorological station located 30 km south of the study site. Records of daily air temperatures, rainfall, and hours of sunshine were obtained from the St. John's meteorological station located 40 km ESE of the study site. Water temperatures were measured at 2- to 3-d intervals at a depth of 1 m, 15 m from the beach. Beach temperatures were continuously monitored from June 8 until July 25, 1979, using temperature probes buried 6 cm below the surface at four locations (one each at high, mid, and low tide and one above the range of tidal influence). These probes were automatically and sequentially scanned with a YSI telethermometer and the outputs were plotted on a multichannel strip chart recorder. On average, 30 temperature readings of 5 min duration were recorded from each probe on each day. Daily beach gravel temperatures for 1978 were predicted from multiple linear regression models describing the relationship between beach gravel temperatures and water temperature, maximum and minimum air temperature, and hours of sunshine at each of the high, mid, and low tide locations in 1979 (Table 5). To correct for departures from normality we arcsin transformed all nonnormally distributed data before regression analysis (Sokal and Rohlf 1969). All values reported in the text are in the untransformed state.

As expected the important variables regulating incubation temperature differed between locations. At the high tide location, which is most exposed, water temperature, maximum and minimum air temperature, and hours of sunlight all contributed significantly to the determination of average daily incubation temperature. At the low tide location, which is least exposed, water temperature alone figured significantly in determining incubation temperature. The situation at the mid tide location was intermediate. At each of these locations >75% of the total variance in average daily incubation temperature was explained by the relevant variable(s) (Table 5).

Average daily incubation temperatures were highly variable and differed significantly between locations on the beach. High tide zone temperatures were consistently 3−5°C

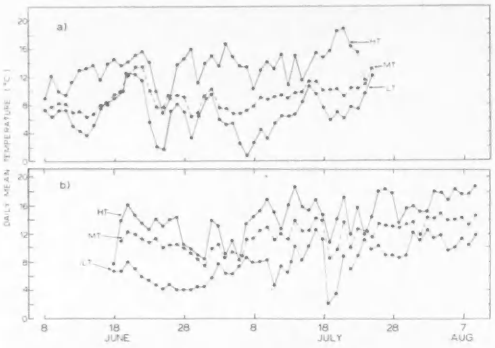


FIG. 3. Mean daily temperature in the beach gravel at the high (HT), mid (MT), and low (LT) tide locations. (a) 1979 observed, (b) 1978 predicted.

higher than those at mid tide and 6−7°C higher than temperatures at low tide (Fig. 3a). Temperature variations over a 24-h period were also large, mean $\Delta T$ and coefficient of variation of mean daily temperature being directly related to the degree of exposure (high tide $\Delta T$ 11.5, CV 28.6; mid tide $\Delta T$ 4.9, CV 21.8; low tide $\Delta T$ 4.4, CV 15.8).

## EGG DEVELOPMENT RATES AND HATCHING TIMES

In describing development rates least square methods were used on variables that were essentially ranked. This was done solely to describe quantitatively the very obvious linear trends in the data. No statistical analyses of differences in slopes or intercepts were performed.

Eighty-five percent of the variability in median hatching times, as determined from extrapolations of field and experimental egg development rate determinations (Fig. 2), was explained by average daily incubation temperatures. This relationship was consistent between years, between sites, and between cohorts (Fig. 4) and is described by the equation $\ln y = 4.27 - 0.63 \ln (x + 1)$, $r^2 = 0.85$, $P < 0.001$, where $y$ = days to medium hatching and $x$ = average daily incubation temperature. The strong similarity in the hatching time relationships between 1979 when incubation temperatures were recorded and 1978 when they were predicted from climatic and water temperature data give added credence to the use of these data in estimating incubation temperatures in future years.

The relationship between hatching time and average incubation temperature is statistically indistinguishable from a relationship we derived using data from a series of experiments reported by Jeffers (1931), Sleggs (1933), and Fridgeirsson (1976) in which capelin eggs were experimentally reared at a series of discrete constant temperatures ($\ln y = 4.29 - 0.63 \ln (x + 1)$, $r^2 = 0.89$, $P < 0.001$; Fig. 4).

The similarity of these two relationships, given the considerable differences in the instantaneous incubation temperatures experienced by eggs in the controlled temperature experiments of Jeffers, Sleggs, and Fridgeirsson and the highly variable conditions in the beach gravel at Bryant's Cove, indicate that capelin egg development rates are largely controlled by the average daily temperature over the incubation period. Griffiths (1978) reported similar findings for "splake" and rainbow trout (*Salvelinus gairdneri*).

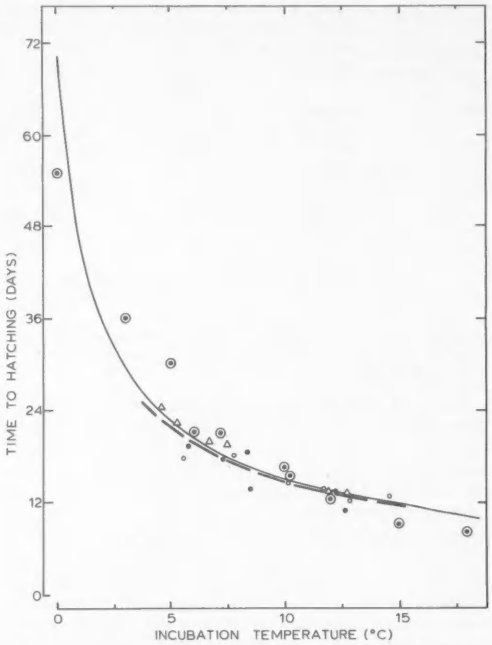


FIG. 4. Relationship between time to hatching and the incubation temperature experienced prior to hatching. Solid line fit to literature data (⊙) describing time to hatching at various constant incubation temperatures. Broken line fit to data obtained from 1978 (○) and 1979 (●) field samples and the development rate experiments (△) conducted in 1979.

The duration of the hatching interval was negatively correlated with average incubation temperature ($y = 4.06 - 0.19x$, $r^2 = 0.93$, $P < 0.01$). As a result the hatching interval was more constrained in time at the high tide location relative to the mid and low tide sites (Fig. 2). Similar negative relationships between hatching intervals and incubation temperatures have been reported for Windermere char, *Salvelinus alpinus* (Swift 1965); brook trout, *S. fontinalis*, and rainbow trout, *S. gairdneri* (Garside 1966), lake whitefish, *Coregonus clupeaformis* (Brooke 1975), and Pacific herring, *Clupea pallasi* (Alderdice and Velsen 1971).

## EGG MORTALITY

In our evaluation of the importance of factors influencing egg mortality we studied the effects of the physical variables described previously and, in addition, we examined the importance of egg density because several variables related to density including oxygen demand and waste product accumu-

TABLE 6. Multivariate models to predict percentage egg mortality ($y$) at the high (HT), mid (MT), and low (LT) tide sites. Untransformed values for $y$ are obtained by $y = (\sin y)^2$.

| Independent variables | Intertidal location | | |
|---|---|---|---|
| | HT slope | MT slope | LT slope |
| Egg no. | −1.26896 | — | — |
| Egg no. × water temp. | — | −0.04070 | −0.03813 |
| Egg no. × max. air temp. | 0.04551 | — | — |
| Egg no. × hours of sunlight | 0.04734 | 0.01474 | 0.04215 |
| Egg no. × tidal amplitude | — | 0.44109 | — |
| Tidal amplitude | — | −22.86834 | — |
| Min. air temp. | — | — | 3.97472 |
| Water temp. × max. air temp. | — | −0.03303 | — |
| Water temp. × hours of sunlight | — | — | −0.15728 |
| Water temp. × daily rainfall | — | — | 0.32235 |
| Water temp. × tidal amplitude | −0.95338 | — | — |
| Min. air temp. × max. air temp. | — | — | −0.09886 |
| Daily rainfall × tidal amplitude | — | — | −2.90611 |
| Max. air temp. × hours of sunlight × min. air temp. | −0.00682 | — | — |
| Intercept | 46.57794 | 48.27720 | 15.33223 |
| $r^2$ | 0.74 | 0.70 | 0.81 |
| $P$ | <0.01 | <0.01 | <0.01 |

lation are known to influence survival in other species (see references in the introduction).

Given that egg mortality in capelin probably results from a whole energy balance effect on the beach rather than from the effect of any single variable operating independently (see previous discussion of relevant variables influencing the beach environment), we grouped the 1978 and 1979 data and, using a stepwise regression approach (STEPWISE/MAXR, SAS Users Guide 1979 Edition), developed empirical models to predict egg mortality at the high, mid, and low tide locations. As initial inputs to the model we employed water temperature, maximum and minimum air temperatures, hours of sunlight, and tidal amplitude plus second-order variables and one third-order variable based on potential interactions between variables. Only those variables contributing significantly to a reduction in the explained variance in egg mortality at the high, mid, and low tide locations, respectively, were retained in the models. The resulting models explained a significant proportion (>70%) of the total variation in egg mortality at all three sites (Table 6). There was also good agreement between the predictions of the models and the observed values for the individual cohorts spawned in each year (Fig. 5). Although we in no way imply that these observations validate the models, the similarity of the observed versus expected mortality for the two cohorts spawned in each year does indicate that the models are statistically appropriate and are capable of accurate predictions of egg mortality over the full range of the data.

Egg density and first-order variables combining temperature and density effects contributed strongly to the explained variance at high tide. These findings suggest a metabolic regulation of mortality at this site. This hypothesis is further strengthened by the strong negative coefficient between egg mortality and the first-order variable water temperature × tidal amplitude. Egg density, temperature, and tidal amplitude variables were also strongly related to egg mortality at mid tide sites suggesting similar, but clearly not identical, metabolic influences at these sites. Egg mortality at low tide sites was more strongly influenced by temperature events and rainfall. At these sites where inundation times are more prolonged, and capillary wetting more likely, direct density effects were small suggesting that oxygen uptake, waste accumulation, or other density-related metabolic factors are less important than direct temperature influences.

The only independent variable identified as a significant regulator of egg mortality at all three sites was egg number × hours of sunlight. Jeffers (1931) reported poor egg survival and a high proportion of deformed larvae when eggs were incubated under conditions simulating bright sunlight. Incubation in total darkness yielded near normal survival and a low incidence of deformities. The constant positive relationship between egg mortality and egg number × hours of sunlight may derive, in part, from the negative effect of high radiation intensity on development. Higher metabolic rates resulting from solar heating may also contribute to the effect of this compound variable.

Simulation of the relative success of the various cohorts of the 1978 and 1979 year-classes spawned at Bryant's Cove suggests that important within year and between year differences in survival to hatching occur as a result of the interaction of biotic and abiotic variables (Fig. 6).

The combined equations describing the influence of meteorological conditions and water temperatures on incubation temperatures, the effect of incubation temperatures on time to hatching, and the effects of meteorological conditions, water temperature, tidal conditions, and egg density on egg survival create the potential for prediction of hatching success in different cohorts or years from easily obtainable data. More

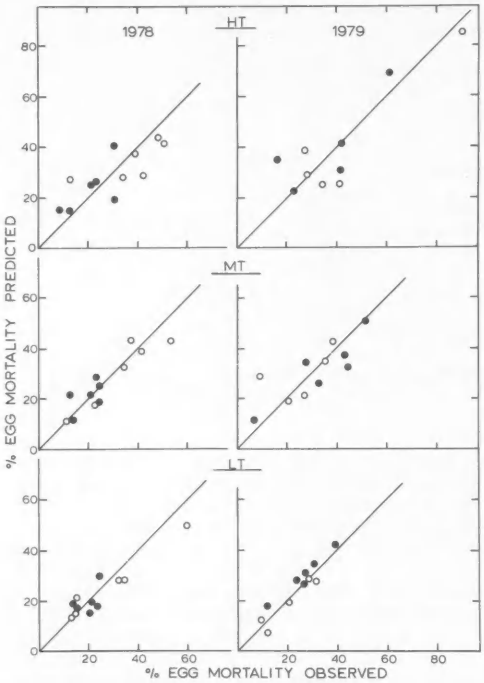


FIG. 5. Observed versus predicted egg mortality for two egg cohorts (cohort 1, ○; cohort 2, ●) at high (HT), mid (MT), and low (LT) tide locations during 1978 and 1979. Predicted values were obtained from the models in Table 6. The line drawn in each panel has a slope equal to one.

importantly, if the timing of egg deposition and meteorological and hydrographic conditions do not vary greatly over the major east coast spawning sites, it may be possible to predict major trends in egg survival over the entire coast. We assessed this possibility by first determining the magnitude of changes in air and water temperatures, rainfall, and hours of sunlight over the east coast of Newfoundland and southern Labrador (47°N to 54°N) and then applying these data to the egg mortality models developed at Bryant's Cove.

Templeman (1948) reported the date of first capelin spawning on beaches over this latitude in 1941 and 1942. These data indicate no relationship between latitude and time of first spawning ($r = 0.19$, $P > 0.25$). We conclude, therefore, that the timing of egg deposition and the period of egg incubation overlaps strongly at all spawning sites over the range. This synchrony of spawning times is expected given the similarity in water temperatures over the range (Templeman 1948; and see below).

Sea surface water temperatures (Canadian Forces METOC Centre, Halifax) for the 5-yr period 1976–80 revealed no meaningful trends in surface temperature with latitude over two separate time periods (June 15–30; July 1–15) encompassing the main incubation dates. Regressions of surface

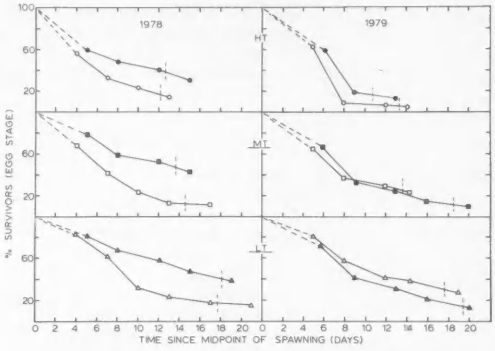


FIG. 6. Simulation of cumulative egg survival to time of median hatching (broken vertical line) at high (HT), mid (MT), and low (LT) tide locations in 1978 and 1979. Predictions based on models in Table 6. (○) First egg cohort, (●) second egg cohort.

temperature versus latitude were significant ($P < 0.05$) only for the July 1–15 period but even in this interval the $\Delta T$ between latitudes 47 and 54 predicted from the equation was <1°C. Maximum and minimum air temperatures (1941–70, Canadian Normals 1973 — Temperature) were negatively correlated with latitude ($P < 0.05$). The average daily minimum temperatures were 3.9 and 4.7°C lower at 54°N relative to 47°N in June and July, respectively. Maximum daily air temperatures were 5.4 and 5.3°C lower in the north over these same intervals. Daily rainfall (1941–70 Canadian Normals 1973 — Precipitation) averaged 1.21 and 0.11 cm higher at 54°N in June and July, respectively. Records of daily hours of sunshine were not adequate for this area; however the number of days having measurable precipitation was independent of latitude (June, $r = 0.217$; July, $r = 0.401$, $P > 0.5$), suggesting an approximately uniform pattern of sunshine.

Egg mortality at 54°N was estimated by modifying the inputs to the models derived at Bryant's Cove on the basis of the maximum and minimum air temperature and rainfall differences derived from the preceding analysis. We assumed that egg densities were similar to those recorded at Bryant's Cove. Egg densities clearly vary between areas and between years. At present the magnitude of these variations is unknown although between year variations appear to be greater than between site variations. Our present analysis thus examines only the impact of geographic variation in meteorological and hydrographic variables on egg mortality. A second assumption was that water temperatures, average hours of sunlight, and tidal amplitude do not differ significantly between latitudes. This assumption was verified above. The resulting simulations (Fig. 7) indicate that differences in survival between 47°N and 54°N, as a result of environmental influences, would be slight. Ten of 12 simulations differed by less than 10%. Survival differences between two cohorts at a single location in a given year can differ by much more than 10% (Fig. 6). The limited differences in predicted egg survival over 7° of latitude, relative to local site differences, lead us to conclude that egg mortality at beaches on the east coast of Newfoundland and southern Labrador may vary synchronously in response to year to year changes in meteo-

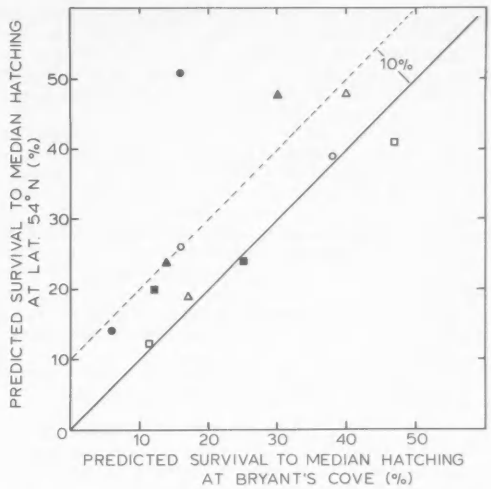


FIG. 7. Relationship between predicted cumulative egg survival to median hatching at 47°N and 54°N at the high (○), mid (□), and low (△) tide sites. Open symbols based on cohorts 1 and 2 in 1978. Closed symbols based on cohorts 1 and 2 in 1979.

rological and hydrological conditions during the egg incubation period. If so, the egg development rate and mortality models developed at Bryant's Cove may have sufficient generality to facilitate yearly estimates of these early life history parameters over the entire coast using as inputs easily obtainable biological, hydrographic, and meteorological data.

## Acknowledgments

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# Relationship Between Destructive Grazing by the Sea Urchin, *Strongylocentrotus droebachiensis*, and the Abundance of American Lobster, *Homarus americanus*, on the Atlantic Coast of Nova Scotia

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WHARTON, W. G., AND K. H. MANN. 1981. Relationship between destructive grazing by the sea urchin, *Strongylocentrotus droebachiensis*, and the abundance of American lobster, *Homarus americanus*, on the Atlantic coast of Nova Scotia. Can. J. Fish. Aquat. Sci. 38: 1339 – 1349.

Relationships between percentage cover of macroalgae, population structure of sea urchins (*Strongylocentrotus droebachiensis*), and the history of the lobster fishery were examined at nine sites distributed along the Atlantic coast of Nova Scotia from Guysborough County in the northeast to Pubnico in the southwest. At Pubnico there was dense algal cover and a small number of urchins living in crevices, and at Cape Sable there was an area of transition, but at all other sites there were urchin-dominated barren grounds. When the population structure of the urchins was compared with that previously observed in St. Margaret's Bay before, during, and after destruction of beds of kelp (*Laminaria* spp. and *Agarum*) by overgrazing, it was concluded that kelp bed destruction occurred on the coast north of Halifax prior to 1970, and on the coast south of Halifax after 1970. From the records of American lobster (*Homarus americanus*) catches it was concluded that a critical decline in catches at each site occurred a few years after kelp bed destruction. Information from various sources is synthesized into a coherent theory of the relationship between lobsters, other predators, sea urchins, and kelp.

*Key words*: lobsters, *Homarus americanus*; sea urchins, *Strongylocentrotus droebachiensis*; destructive grazing, kelp, *Laminaria*, *Agarum*

WHARTON, W. G., AND K. H. MANN. 1981. Relationship between destructive grazing by the sea urchin, *Strongylocentrotus droebachiensis*, and the abundance of American lobster, *Homarus americanus*, on the Atlantic coast of Nova Scotia. Can. J. Fish. Aquat. Sci. 38: 1339 – 1349.

Nous avons examiné, à neuf sites répartis le long de la côte atlantique de la Nouvelle-Écosse, entre le comté de Guysborough au nord-est et Pubnico au sud-ouest, les relations entre le pourcentage de couverture de macro-algues, la structure de population de l'oursin commun, *Strongylocentrotus droebachiensis*, et l'historique de la pêche du homard. À Pubnico, il y a dense couverture algale et un petit nombre d'oursins habitant les crevasses. Au cap Sable, se trouve une aire de transition, alors qu'à tous les autres sites, ce sont des fonds dénudés, dominés par les oursins. Une comparaison de la structure de population d'oursins avec ce qui avait déjà été observé dans la baie St. Margaret avant, pendant et après la destruction des lits de varechs (*Laminaria* spp. et *Agarum*) par broutage excessif, a permis de conclure que la destruction des lits de varechs se produisit sur la côte, avant 1970 au nord et après 1970 au sud de Halifax. À l'examen des statistiques de prises de homard américain (*Homarus americanus*), nous concluons qu'une diminution critique des prises à chaque site s'est produite quelques années après la destruction des lits de varechs. L'information provenant de diverses sources a été synthétisée en une théorie cohérente de la relation entre le homard, les autres prédateurs, l'oursin commun et les varechs.

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THE idea that many complex communities with a multitude of feeding interactions are effectively structured by a relatively small number of strong interactions is now gaining wide acceptance. Paine (1980) has recently reviewed the subject, giving examples from his own pioneering efforts and from the work of others. It seems to be the case, at least in aquatic communities involving macrophytes, that changes in the population density of a key predator can cascade down through the system to produce major shifts in the balance of primary producers. Duggins (1980), building on the work of others, has now shown conclusively that in Alaska sea otters are key predators on sea urchins (*Strongylocentrotus* spp.), and that where otters are abundant sea urchins are relatively scarce but kelps flourish. Conversely, where sea otters are scarce, urchins are more abundant and consume most of the macroscopic algae. This paper documents a parallel case where sea urchins have a major structuring effect on the algal communities of Eastern Canada and discusses American lobster (*Homarus americanus*) stocks from two points of view: as possible key predators but also as targets for a positive feedback in the system, so that loss of macroscopic algae leads to still further declines in lobster populations.

In a series of papers recording observations and experiments over more than a decade, it was established that in St. Margaret's Bay, Nova Scotia, the kelp beds (*Laminaria longicruris*, *L. digitata*, and *Agarum cribrosum*) were almost 10 times as productive per unit area as the phytoplankton in the bay, but that sea urchins *S. droebachiensis* were capable of forming destructive grazing aggregations which produced areas of rock completely devoid of macrophytes. These areas, at first relatively small and temporary, became progressively larger and more permanent until they coalesced and most of the kelp beds in the bay were destroyed (Mann 1972a, b, 1973, 1977; Miller and Mann 1973; Breen and Mann 1976a). The hypothesis was put forward (Mann and Breen 1972) that the cause of the phenomenon was release of predation pressure on the urchins, caused by overfishing of lobster stocks. Direct proof of this hypothesis was lacking but a model showed it to be consistent with the population dynamics of urchins and lobsters (Breen and Mann 1976b) and experimental studies of the feeding of lobsters showed that although sea urchins were not the most preferred prey, the lobsters would nevertheless eat large numbers of urchins if these were readily available (Evans and Mann 1977; Hirtle and Mann 1978). Bernstein et al. (1981) noted that fish played a role in dispersing urchin aggregations and there might well be two stages in the process: first, a buildup of urchin density within the kelp beds, probably due to a reduction in predator pressure from invertebrates living inside the beds, and second, formation of destructive grazing aggregations which persist only if they are not destroyed by fish predation.

This paper carries the argument forward in two directions. It shows first of all that urchin-dominated, macrophyte-free areas are not confined to St. Margaret's Bay, but extend for more than 500 km along the Atlantic coast of Nova Scotia. Second it shows that there is a correlation between kelp bed destruction and falling lobster catches. The hypothesis is put forward that, regardless of the initial cause of urchin population increases, destruction of kelp beds leads to a decrease in lobster productivity on account of (i) loss of cover, which increases the exposure of young lobsters to their predators, and (ii) loss of productivity in the food web.

A workshop sponsored by Fisheries Management personnel of the Canadian Government's Department of Fisheries and Oceans met in Halifax in March 1979 specifically to consider the ideas on kelp–urchin–lobster interactions summarized above. To provide a focus for this paper, and to enable the reader to form his own opinions, the six workshop conclusions (Pringle et al. 1980) are given in full.

> 1) For some areas, a significant decline was observed in macrophyte biomass over the last decade; for many other areas, base-line data are not available but circumstantial evidence (mainly anecdotal) suggests there has been a general decline on the Nova Scotia shore east of Cape Sable Island.
>
> 2) In areas where destructive grazing of macrophytes by sea urchins has been observed, they are the direct cause of macrophyte biomass decline. The data available are insufficient, however, to claim they are the sole cause, as there have been environmental changes.
>
> 3) There has been an increase in the biomass of sea urchins at specific sites on Nova Scotia's coast where data are available, but data are insufficient to claim an increase in the density of urchins along vast stretches of Nova Scotia's coast.
>
> 4) Based on anecdotal evidence only, there appears to be a degree of cyclicity between high sea urchin densities and high kelp densities in certain areas of the Maritime coast. The length of the period of the "urchin-dominated barren" mode in St. Margaret's Bay is longer than the others cited, but this does not imply permanency.
>
> 5) Overfishing appears to have been a major factor in the decline of lobster landings along Nova Scotia's eastern shore. There is little evidence environmental factors, lack of food, or lack of plant cover were important factors in this decline.
>
> 6) Evidence at present is insufficient to claim lobsters are the prime predators of sea urchins and that urchin populations in St. Margaret's Bay exploded due to the release from predation by the lobsters.

The dilemma that confronts us is but one of a very common class of dilemmas confronting ecologists. Ecosystems are complex, and an enormous amount of work is required to demonstrate the validity of every interaction in any particular system. By exercising a strong degree of scepticism about an ecosystem theory one can almost always demonstrate that the ecological evidence is insufficient and that a verdict of "not proven" is justified. Meantime that ecosystem may be deteriorating and a valuable resource may be lost before a fully acceptable explanation has been found.

The purpose of this paper is to draw attention to the deteriorating condition of the lobster-producing system of Atlantic Nova Scotia and to attempt to change the concensus of management personnel from "not proven" to something like "seems plausible, worth investigating further."

## Sites and Methods

Nine sites along the coast of Nova Scotia were selected for intensive study in 1978 and 1979 (Fig. 1). Each was an area

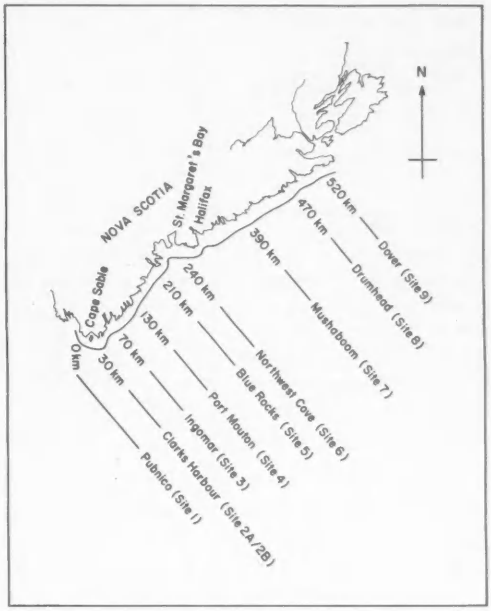


FIG. 1. Sketch map of the Atlantic Coast of Nova Scotia, showing nine study sites and distance from Pubnico. (See Fig. 7 for further details.)

for which good lobster catch statistics were available and was judged free of anomalous conditions such as serious pollution, which might have affected the distribution of kelp and urchins. At each site, the study area was defined as the fishing area of the inshore lobster fleet of the nearest port or group of ports. The maximum number of sites that could have been separately investigated is 13, which is the number of "statistical districts" for lobster records. Four sites were rejected as polluted or otherwise perturbed.

Because the greatest biomass of macroalgae occurs from the shoreline out to a depth of 20 m (Mann 1972a) and because the distribution of lobster traps when fishing is also mainly in this zone, a sampling scheme was arranged which centered on the 10-m contour. Each study area was divided into squares of 0.5 × 0.5 nautical miles (0.93 × 0.93 km) on a hydrographic chart and all squares on the 10-m contour were considered for sampling. They were numbered serially and choice of square was made from a table of random numbers. The procedure for sampling a square was to move out from shore (or a shoal) and drop a marker at the first place the 10-m contour was encountered. From this marker, three 50-m transects were laid out on the bottom at 120° to each other, with one pointing to shore or shoal. At each 1-m mark on each transect, the substrate was classified as suitable or unsuitable for the growth of macroalgae, rocks over 10 cm in diameter and boulders being suitable. If the substrate was suitable, a record was made of the presence or absence and the identity of any macroalgae (other than corallines) present.

Near the center marker, four to eight quadrat samples of sea urchins were taken, using a 56.4-cm line, weighted at one end, which was dropped randomly and used to define a circle of area 1 $m^2$. All urchins were preserved and later had their diameters measured with vernier calipers. A subset was aged by a modified method of Jensen (1969). A piece of an interambulacral zone was cut from the test, cleaned in 95% alcohol with a soft toothbrush, immersed in 3% sodium hypochlorite solution for 1 h, air-dried for 12 h, charred over an alcohol flame until the plates were light brown, cleared in xylene, and examined under a dissecting microscope. Age was recorded as the number of dark summer growth rings on plates.

At each of the nine sites the sampling process was repeated at 10–15 randomly selected squares. For each square, the mean percentage of algal cover, urchin density, urchin diameter, and urchin size at each age were calculated. Grand means were calculated for each site. The data from Clark's Harbour (site 2) were found to fall into two quite different sets, so the results have been presented as sites 2A and 2B. For significance testing and display, all algal cover data were subjected to arcsin transformation.

Lobster catch data were obtained from Caddy and Chandler (1976) and from the records of the Statistics Branch of Fisheries and Oceans Canada. The data used were for the statistical areas in which the study sites occurred. The statistical areas were larger than the study sites, but by discussion with fishermen and fishery officers it was ascertained that the study sites yielded a major component of the catch of the statistical areas in which they occurred.

The mean values for the years 1947–56 (the earliest years for which complete records for those areas are available) were used as reference values and recent catches were expressed as percentages of that 10-yr mean.

## Results

### VARIATION IN KELP DISTRIBUTION

From algal abundance data we can distinguish two major community types, "established kelp" and "urchin-dominated barren grounds." A similar distinction was made in Alaskan kelp beds by Duggins (1980). Figure 2a shows the percentage algal cover at the nine sites along the Nova Scotia coast. For convenience of display, the horizontal axis is given as the distance along the coast from site 1 at Pubnico. At Pubnico and at site 2A at Clark's Harbour, there was a very high percentage cover by macrophytes. These were the established kelp communities. They had dense growths of *Laminaria longicruris* and *L. digitata*, with smaller amounts of *Agarum cribrosum* and *Alaria esculenta*. They extended from low water mark to a depth of ~20 m in a zonation pattern similar to that described by Mann (1972a).

At Ingomar (site 3) and at all sites further from Pubnico, the mean algal cover was never above 20% and the communities present were of the type described by Lawrence (1975) as "urchin-dominated barren grounds." What little algal cover was encountered was either situated in areas of high wave action (e.g. tops of high rocks) or consisted of the relatively unpalatable species *Desmerestia viridis* and *A. cribrosum* (Paine and Vadas 1969).

At Clark's Harbour (site 2) there was an area of dense algal cover (site 2A) and one where the cover was around 50% (site

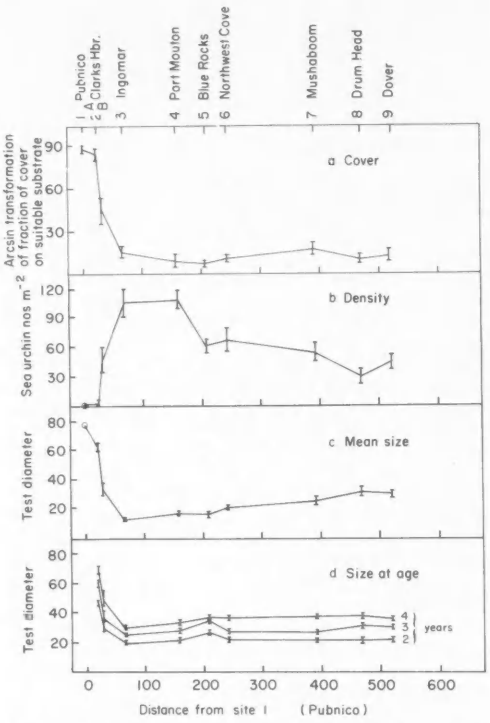


FIG. 2. Data on kelp cover and urchins plotted along the axis "distance from Pubnico." (a) Percentage cover by macroalgae (after arcsin transformation); (b) population density of urchins; (c) mean size of urchins; (d) size of urchins of ages 2, 3, and 4 yr. Figure shows that near Pubnico kelp cover is dense and urchin populations are sparse, but much of the remainder of the coast is "urchin-dominated barren grounds."

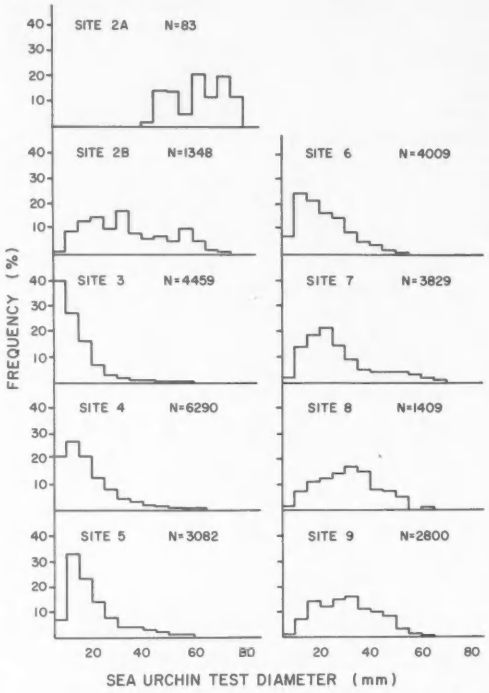


FIG. 3. Size–frequency distribution of urchins at sites 2A–9. Too few urchins were found at site 1 to permit a plot of size distribution. $N$ = number of urchins measured. Note that sites 3–6 are dominated by small, young urchins, but sites 7–9 are dominated by larger urchins.

2B). The latter may be described as a transition zone as its condition was intermediate between that of established kelp and that of the urchin-dominated barren grounds. There were aggregations of sea urchins, some in irregular clumps and some in more linear "fronts," so that site 2A resembled areas of active kelp bed destruction described in Mann (1972a) and in Breen and Mann (1976a).

## Variation in Sea Urchin Population Characteristics

Figure 2b shows that the density of sea urchins within established kelp (site 1 and site 2A) was very low. The values were 0.4 $m^{-2}$ at site 1 and 4.0 $m^{-2}$ at site 2A. With increasing distance from Pubnico, sea urchin densities increased, exceeding 100 $m^{-2}$ at sites 3 and 4, but then declined to densities of 40–60 $m^{-2}$ at the three stations furthest from Pubnico.

Mean size of urchins appeared to be inversely related to population density (Fig. 2c). In dense kelp, where urchins were not abundant, the mean size was 60–80 mm diameter but at the sites of high urchin abundance the average diameter was under 20 mm. Moving further from Pubnico, the mean diameter increased slowly to about 30 mm.

Size–frequency distribution (Fig. 3) changed in parallel with other parameters. It is clear that in areas of established kelp beds where there were few urchins, most of those present were relatively large. At sites 3 and 4, where population density was highest, there was an abundance of small urchins, indicating the presence of recent, highly successful year-classes of recruits. In sites further from Pubnico, the dominant year-classes were seen to be in progressively older age groups.

The plots of mean size of each age group (Fig. 2d) show that when urchin-dominated barren grounds are compared with established kelp communities the urchins of a particular age on the barren grounds are no more than half the size of those in the kelp, indicating a greatly reduced growth rate. The same reduction in growth rate was reported for urchins on barren grounds in St. Margaret's Bay by Lang and Mann (1976).

## Variation in the Catch History of the American Lobster

Figure 4a shows the catch data for the relevant statistical areas for 1977, 1978, and 1979 expressed as a percentage of

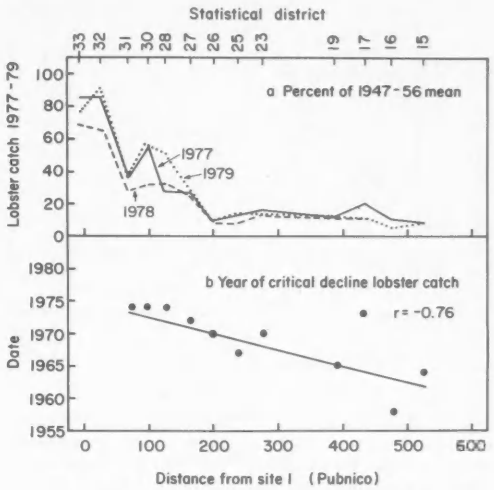


FIG. 4. Data on lobster catches plotted along axis "distance from Pubnico." (a) Catches of lobsters in 1977, 1978, and 1979 expressed as a percentage of the 10-yr mean for 1947–56. Statistical districts from which data were obtained are shown on upper edge of diagram. (b) Year in which lobster catch declined to below 60% of the 10-yr mean, and never recovered. Correlation coefficient between date of critical decline and distance from Pubnico, $r = -0.76$.

the 10-yr mean for 1947–56. In every case the catches were less than 100%, but they were relatively high in the areas of established kelp (sites 1 and 2A) and relatively low in the urchin-dominated barren grounds, particularly those furthest from Pubnico.

When we examined the time series of catch data, we found that sharp declines in catch had occurred, first at the sites furthest from Pubnico and more recently at sites closer to Pubnico. As an index of this time series, we arbitrarily identified a year of "critical decline" as the year when the catch fell below 60% of the 1947–56 mean and did not recover. The date of this decline is given in Fig. 4b and it is clear that it occurred earlier at the sites more remote from Pubnico.

### Intensive Study of the Transition Zone

The original study of sites 2A and 2B was undertaken in the summer of 1978. During the fall and winter of 1979 it was possible to carry out an intensive reexamination, as part of a contract with the Canadian Government (DSS Unsolicited Proposal #LSC 79-00058). The Scientific Authority for this contract was Dr. J. Pringle, Head of Marine Plants Section of the Department of Fisheries and Oceans, Halifax. A full analysis has not yet been published but the following general results are quoted with permission. In the region of sites 2A and 2B, 28 transects perpendicular to shore were sampled quantitatively. In addition, qualitative estimates were made by divers drifting through the area with prevailing currents. In 1978 area 2A was dominated by *Laminaria* but by 1979 large parts of it had had *Laminaria* removed by selective grazing, leaving a sparse cover of *Agarum*. In the areas still having *Laminaria*, urchin density averaged 0.66 $m^{-2}$ but in *Agarum*-dominated areas it was 2.66 $m^{-2}$. It appeared that kelp destruction was occurring by urchin infiltration and selective grazing, rather than by the formation of fronts as described by Mann and Breen (1976a). A few such fronts were found, but only near the boundary between areas 2A and 2B. The preference of urchins for *Laminaria* rather than *Agarum* was first demonstrated by Vadas (1968), who also showed that gonad production was closely related to the quality and quantity of food available.

In area 2B the macrophyte cover was estimated at 49% in 1978 but only 20% in 1979, suggesting that kelp destruction was occurring rapidly at Clark's Harbour between 1978 and 1979. Because the sampling procedures were entirely different in the two surveys, no rigorous statistical comparison can be made and the results must be taken as indicative only of a trend.

## Discussion

### Hypothesis

The interpretation that we offer for our results is first given in outline, and the arguments in support of it follow. Our hypothesis is that the kelp cover along the outer coast of Nova Scotia was once much more dense than it is now, and in that kelp the density of urchins was quite low, of the order of 1 $m^{-2}$ (as it now is in the Pubnico area). Beginning at the northeast end of the province, urchin numbers began to increase. When they reached 2–10 $m^{-2}$ they began to overgraze the kelp, producing urchin-dominated barren grounds. The barren grounds appeared first in the northern half of the province and later spread closer to Pubnico until they reached Clark's Harbour, where kelp bed destruction appears to be active at present.

Within a few years after each area was cleared of kelp, there was a very successful recruitment to the urchin population bringing the numbers up to somewhere near 100 $m^{-2}$ and reducing the mean size of urchins present. As the years went by, the successful year-class moved up through the size groups and the population density decreased to around 60 $m^{-2}$, then to around 40 $m^{-2}$. The growth rate declined because these populations were severely food limited. Hence along the coast we may recognize several stages in a time series: (i) healthy kelp with low urchin density (site 1), (ii) kelp with higher urchin density (site 2A), (iii) kelp in the process of destruction by urchin grazing (site 2B), (iv) urchin-dominated barren grounds with large numbers of small urchins (sites 3 and 4), (v) urchin-dominated barren grounds with fewer urchins and a slightly larger mean size (sites 5–9). There is at present no way of knowing directly how long ago the kelp destruction began, except that it has been recorded (Breen and Mann 1976a) that in St. Margaret's Bay, close to site 6, kelp destruction had begun in 1968 and was most active from 1972 to 1975.

When an area is converted from kelp bed to barrens there are two types of change that could be expected to affect lobsters: loss of productivity and loss of habitat. We believe that on barren areas the lobsters found fewer invertebrates suitable for food and that the absence of kelp made the young lobsters more vulnerable to predators, hence decreasing re-

cruitment to the fishable stock. With a lag of a few years, these changes have led to a decrease in lobster landings. Because the critical decline in lobster landings occurred prior to 1965 near stations 8 and 9, between 1965 and 1970 at stations 5 and 6, and between 1970 and 1975 near station 4, we think that there is good evidence for a decline in lobster landings in a time series that parallels the events in kelp–sea urchin interactions. Lobster landings in the area of stations 2 and 3 are not yet as low as at stations 4–8, indicating a time lag between kelp bed destruction and collapse of the lobster stocks. Statistical district 17 (between stations 7 and 8) experienced catches just below or just above the 60% level for several years, and this appears to be out of line with the trend described above.

The final part of our hypothesis concerns the mechanism which initiates kelp bed destruction by urchins. Noting that in an intact kelp bed at Pubnico there were very few urchins but that in the kelp close to the transition zone at Clark's Harbour the density was about 50 $m^{-2}$, we suggest that the increase in urchin density that occurs prior to urchin overgrazing is caused by reduced predation pressure. The important predators include lobsters, crabs, and various fishes, but we have as yet no direct evidence to enable us to identify the species whose decline led to the population increase of urchins. Nevertheless, when the natural history of the various predators is taken into account, it seems probable that environmental changes plus fishing pressure initiated a decline in lobster stocks just at the time that urchin numbers began to increase, and that reduction in predation pressure from lobsters was the immediate cause of that increase. As will be shown, reduction in fish predation was also necessary to permit destructive grazing.

## Kelp Cover in Times Past

The hypothesis outlined above rests on the assumption that the Atlantic coast of Nova Scotia once had dense algal beds and that the destruction of these took place within the last 25 yr. In the areas that have been thoroughly investigated, this has proved to be true. Edelstein et al. (1969) reported on transects on the outer coast to the north and south of Halifax, and on spot dives off the mouth of Halifax Harbour, and indicated that in 1965–66 there was a rich and abundant algal flora on all suitable substrates. A total of 166 species was recorded (excluding crustose corallines). From the sublittoral fringe to a depth of 15 m, *Laminaria* and *Desmerestia* were dominant. From 10 m downwards, *Agarum* and *Ptilota* became more abundant, and dominated beyond 15 m. A very similar pattern of zonation and dense cover was reported from St. Margaret's Bay in 1968 (Mann 1972a).

The results of conversations with lobster fishermen up and down the coast support the view that the kelp cover was once much more extensive than at present. Older fishermen frequently talk of dense kelp beds existing in times past, of invasions by urchins being associated with declining catches of lobsters, and of kelp beds being the places "where lobsters like to hide." A typical case history goes something like this: A fisherman recalls that on a particular shoal, after several years of good fishing the lobster catches fell to very low levels and the traps were filled with urchins. Looking down when the water was clear, bare, light-colored rock could be seen, instead of dark brown kelp. The area was left unfished for a few years, the kelp came back, and the lobster catch went up again. The pattern was repeated in various places at different times. The effect was always confined to an area measuring a few hundred metres in diameter and there was always recovery after a period of abstaining from fishing. When the fishermen saw areas of bare rock getting bigger and bigger, and showing no sign of return to normal, this was a new experience.

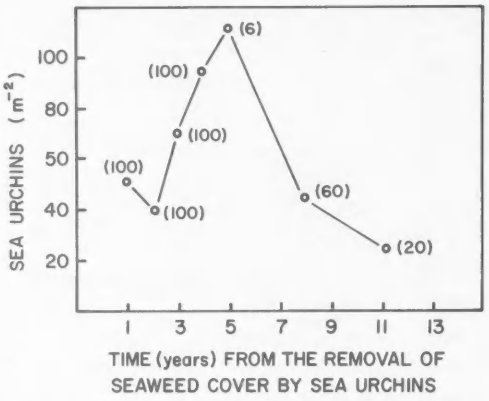


FIG. 5. Composite diagram indicating changes in sea urchin density in St. Margaret's Bay with time from kelp bed destruction. Data for years 1–4 from Lang and Mann (1976), year 5 from Breen (1974), year 8 from Wharton (unpublished data), year 11 from Bernstein et al. (1981). Numbers in parentheses are sample sizes.

## Interpretation of Variation in Sea Urchin Populations

Our interpretation of the spatial variation in sea urchin population parameters rests on the sequence of events observed in St. Margaret's Bay from 1968 to the present. When first sampled in 1968, St. Margaret's Bay had extensive kelp cover but there were limited areas in which sea urchin density was high and macroalgae had been cleared (Mann 1972a). Average urchin density in this system was given as 36.8 $m^{-2}$ (Miller and Mann 1973), but reexamination of the data shows that it was higher in the barren patches and lower ($<10$ $m^{-2}$) in the intact kelp beds. Lang and Mann (1976) returned to sites where kelp had been destroyed 1, 2, 3, and 4 yr previously. Breen (1974) reported on a site cleared 5 yr earlier, Wharton (unpublished data) studied a site 8 yr after kelp disappearance, and Bernstein et al. (1981) studied the same area 11 yr after kelp destruction. A composite diagram showing changes in urchin density (Fig. 5) indicates an increase to about 100 $m^{-2}$ within the first 4–5 yr, followed by a decline to under 30 $m^{-2}$. Changes in population size structure (Fig. 6) indicate that the increase in population density was caused by heavy recruitment of young urchins, after which recruitment declined and the mean size and age of the urchins in the population increased.

This pattern in time in St. Margaret's Bay bears a striking resemblance to the pattern in space from site 2 to site 9 along

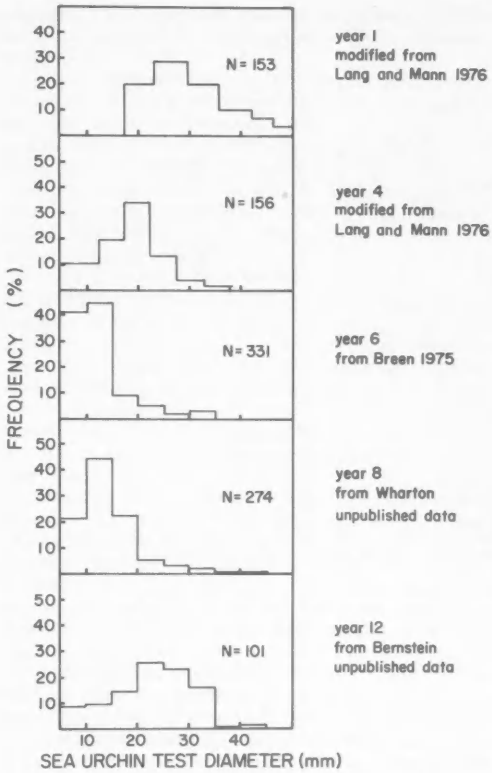


FIG. 6. Size–frequency distribution of urchins in populations in St. Margaret's Bay in various years after kelp bed destruction. Sources of data indicated on right of diagram. Note that, as in Fig. 3, there is a shift from a larger mean size to dominance by small urchins, and finally a return to dominance by a larger size group.

the Atlantic coast of Nova Scotia. Samples of urchins from the area of active grazing at site 2 most closely resemble those from the sites in St. Margaret's Bay soon after kelp bed destruction. Populations of urchins at sites 3, 4, and 5 are similar to those found in St. Margaret's Bay 12 yr after kelp bed destruction. Although it is unlikely that kelp bed destruction took place as a simple frontal movement down the coast (we know, for example, that it took the form of a mosiac in St. Margaret's Bay), our results suggest that the peak of urchin grazing was over 10 yr ago on the coast northeast of Halifax, and less than 10 yr ago southwest of Halifax.

In St. Margaret's Bay we observed the movement of the grazing fronts and showed experimentally that removal of urchins led to the reestablishment of kelps (Breen and Mann 1976a) so that there is no doubt that the urchins were the cause of the kelp disappearance. For other parts of the coast the direct observations of grazing have not been made, although, as mentioned previously, numerous lobster fishermen have described the simultaneous disappearance of kelp and appearance of sea urchins.

## INTERPRETATION OF FALLING LOBSTER CATCHES

If we accept the idea that much of the sublittoral zone of the Atlantic coast of Nova Scotia was once covered by dense growths of macroalgae and now consists of "urchin-dominated barren grounds," what are the probable consequences for the lobster populations? We have abundant anecdotal evidence from lobster fishermen that where kelp beds are present, these are the best places to catch lobsters; in other words, dense algal beds are the preferred habitat of lobsters on this coast. Lobster biologists who have worked extensively in the Gulf of St. Lawrence and the Bay of Fundy may have difficulty in accepting this point of view, but the ecology of lobsters on the rocky shores of the Atlantic coast is different from the ecology of lobsters in less exposed areas. For example, Cooper and Uzmann (1980) recognize "sand base with rock" and "bedrock base with rock" as distinct habitat types for lobsters. The former is more characteristic of Gulf of St. Lawrence and Fundy habitats, and less likely to support dense growths of algae than is the latter. Published evidence is lacking, but it was evident to divers in the 1960s and early 1970s that there was a correlation between the distribution of lobster traps and the distribution of kelp on the Atlantic coast of Nova Scotia.

It seems probable that inhabiting algal beds confers two main benefits on lobsters: (i) shelter from predators and (ii) abundance of food organisms. That shelter from predators has the effect of decreasing mortality is almost self-evident and has been demonstrated in experimental systems many times, for example, by Gause (1934) for Protozoa and by Ivlev (1961) for invertebrates preyed upon by fish. We therefore conclude that removal of the kelp cover by overgrazing probably had the effect of making lobsters move vulnerable to predators. According to Cooper and Uzmann (1980) the major predators on juvenile lobsters are various species of fish, sculpin (*Myxocephalus* spp.), cunner (*Tautoglabrus adspersus*), tautog (*Tautoga onitis*), and sea raven (*Hemitripterus americanus*).

Chapman (1981) documented the reduction in primary productivity of urchin-dominated barrens compared with an intact kelp bed. He found that the biomass of diatoms and other microalgae on the barrens average 2.2 g $C \cdot m^{-2}$ with an annual production of 12.7 g $C \cdot m^{-2} \cdot yr^{-1}$. A nearby kelp bed had a biomass of 185 g $C \cdot m^{-2}$ and a productivity exceeding 1000 g $C \cdot m^{-2} \cdot yr^{-1}$. Hence, he concluded that kelp removal led to a reduction of primary production by two orders of magnitude. It seems inevitable that this should be translated into reduced productivity by the invertebrates that form the food of lobsters. The invertebrate population on the barrens is dominated by sea urchins having a very low growth rate and minimal gonad production (Lang and Mann 1976), so they are a poor food source for predators. Crabs, which are a preferred food source for lobsters (Evans and Mann 1977), are particularly abundant (0.2–0.4 $m^{-2}$) in kelp beds (Drummond-Davis 1978). Hence, we conclude that the removal of kelp probably has the effect of reducing the food supply available to lobsters.

From the two lines of reasoning given above, it appears probable that kelp destruction by sea urchins would lead to a decrease in lobster stocks on account of increased mortality of the juveniles and a reduction in food supply. This is our

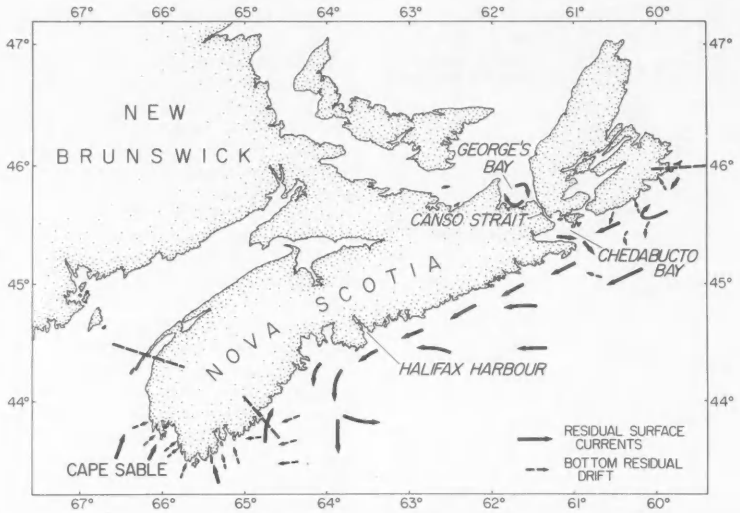


FIG. 7. Surface and bottom residual currents along the Atlantic Coast of Nova Scotia during summer and fall. Dotted lines show boundaries of hypothetical current cells within which lobster larvae are transported. After Dadswell (1979).

explanation for the similarity of the pattern in kelp bed destruction and falling lobster catches. However, other explanations for decreasing lobster catches in eastern Nova Scotia have been put forward, notably by Dadswell (1979) and Robinson (1979). Dadswell developed the extremely interesting hypothesis (Fig. 7) that recruitment to the lobster stocks on the Atlantic coast of Nova Scotia depends on a supply of lobster larvae from upstream in the prevailing current system which runs northeast to southwest along the coast. Before the closure of the Canso Strait by a causeway in 1955, lobster larvae from George's Bay were carried by currents through the strait to provide a considerable proportion of the recruitment in Chedabucto Bay. Approximately one "lobster-generation-time" after closure, i.e. between 1962 and 1964, there was an abrupt decline in catches in Chedabucto Bay. Dadswell (1979) further postulated that the stocks in Chedabucto Bay were the source of larvae for stocks to the southwest and that there was a "domino effect," with the catches between Chedabucto Bay and Halifax dropping rapidly during the period 1965–73 and those southwest of Halifax in the late 1970s.

Robinson (1979) emphasized "recruitment overfishing" as the prime cause of declining lobster landings on the Atlantic coast of Nova Scotia. He showed that under the current management practices, lobsters are subject to heavy fishing mortality prior to attaining sexual maturity and suggested that this factor, together with the increased fishing pressure resulting from improved technology, has led to the observed collapse of the stocks. Although not denying that overfishing (and perhaps the closure of the Canso Causeway) were factors initiating the decline in lobster stocks, we consider that destruction of kelp beds must also have been a contributory factor, through loss of cover and loss of productivity.

## POSSIBLE CAUSES OF SEA URCHIN POPULATION EXPLOSIONS

If, as we have suggested, Fig. 2b can be read from left to right as it is were a time series, the events leading to kelp disappearance and eventually to decline in lobster stocks began with an increase in population density of sea urchins. The idea that intact, healthy kelp beds have very low population densities of urchins receives support from the work of Bernstein et al. (1981). A careful study was made from 1977 to 1980 of a situation in St. Margaret's Bay in which a patch of kelp several thousand square metres in extent had persisted as a remnant of the extensive beds reported in Mann (1972a). Adjacent to the bed was an area of urchin-dominated barrens, and between the two was a 2-m-wide edge zone in which feeding aggregations of urchins appeared from time to time. Bernstein and co-workers found that on the barrens the urchins were present at relatively high densities (20–70 $m^{-2}$); in the edge zone they varied from 5 $m^{-2}$ in 1977 to about 100 $m^{-2}$ in late 1978 and back to about 5 $m^{-2}$ by mid 1979. Within the kelp bed, however, a few urchins were found in 1977 but none were found after that. Field observations suggested that there were sufficient predators (lobsters, crabs) living in the kelp beds to prevent urchins ever becoming abundant. Conversely, it was argued that any marked increase in urchin population density within kelp beds must have resulted from a reduction in predator pressure.

Mann and Breen (1972) first put forward the hypothesis that lobsters were the chief predators responsible for controlling sea urchin numbers and that population explosions of urchins resulted from reduction of lobster populations below a critical level. Breen and Mann (1976b) drew attention to the possibility that other predators, such as wolffish (*Anarrhichus lupus*) and crabs (*Cancer irroratus*), were also important

predators. The results of numerical modeling suggested that the fish and crabs had an effect that was probably subsidiary to that of the lobsters.

The work of Bernstein et al. (1981) has tended to confirm the original idea but to add details which show how complex and subtle are the interactions involved. Fish that prey on sea urchins tend to operate round the edges of kelp beds, but not inside them. The important predators within the beds appear to be lobsters and crabs. They actively consume urchins that settle and develop within kelp beds, and the only urchins that survive there live in crevices inaccessible to the predators, feeding mainly on particles of detritus. Reduction of predator pressure leads to an increase of urchin abundance and at some critical population density the urchins change their behavior patterns, coming out on tops of the rocks and forming dense aggregations which the crustacean predators cannot penetrate. These aggregations move about in the kelp, destroying all plants in their path. When a substantial area has been cleared and the urchins no longer encounter kelp, the aggregations disperse in search of food. Because the crustacean predators tend to stay within the kelp, the urchins are now less vulnerable to them.

The strategy for avoidance of fish predators now becomes important. Bernstein et al. (1981) observed that wolffish and plaice (*Hippoglossoides platessoides*) were significant urchin predators and that their activity was closely correlated with temperature ($r = 0.92$). At 5°C the number of predatory fish observed was very low indeed, and below that temperature none were seen. In summer when fish were active the urchins avoided predation by staying under the rocks by day, but coming out on top of the rocks to feed at night. In winter when fish were less active the urchins tended to stay on top of the rocks day and night. Bernstein et al. (1981) concluded that the survival of the isolated patch of kelp in St. Margaret's Bay was attributable to the activities of the fish in summer, breaking up the aggregations by their predation. The converse of this hypothesis is that kelp bed destruction is a two-step process. Stage 1, buildup of urchin density within a kelp bed, depends on there having been a reduction in predation pressure by invertebrate predators, primarily lobsters and crabs. This leads to stage 2, formation of destructive feeding aggregations, which persist only if they are not destroyed by fish predation.

We thus come to the idea that kelp bed destruction occurs when there has been reduction in predation pressure by both crustaceans and finfish. The overfishing of lobsters described by Robinson (1979) and the construction of the Canso Causeway, discussed by Dadswell (1979), may well have been contributors to the reduction of population density and predation by lobsters. We have no evidence to suggest changes in predation pressure by crabs. There is some evidence (J. Caddy, F.A.O. Rome, personal communication, cited in Pringle et al. 1980) that wolffish stocks have decreased markedly in the last 20 yr, and their role now appears to be complementary, rather than subsidiary, to that of lobsters and crabs.

## Kelp Bed Destruction Accentuates the Decline in Lobster Stocks

It now seems possible to unite the standard hypotheses about recruitment and overfishing of lobster stocks with the broader ecological views described above. Given an initial decline in lobster population density, caused by overfishing and recruitment failure, and given that lobsters are an important (but not the only) predator of sea urchins (Evans and Mann 1977), we suggest that urchin density could increase in kelp beds to the level where the urchins are forced to leave their hiding places and form defensive aggregations on the tops of the rocks. This in turn would lead to destructive grazing. From the evidence of fishermen, we believe that localized outbreaks of destructive grazing have occurred many times in the past. However, the fishermen frequently talked of return to dense kelp cover within 3–5 yr. The agent of recovery may well have been predatory fish, especially wolffish.

If the increase in urchin density also coincided with a period of reduced density of predatory fish, localized areas of destructive grazing would persist, spread, and eventually coalesce. This is what we observed to happen in St. Margaret's Bay between 1968 and 1975. We believe that similar events occurred between Canso Strait and Halifax in the 1960s and between Halifax and Cape Sable (Clark's Harbour) during the 1970s.

If, as we have suggested, loss of kelp cover and productivity leads to further declines in lobster growth and recruitment, the production of lobsters enters a further turn of the downward spiral. In other words, kelp bed destruction is a positive feedback in the system. Once this has happened, even a drastic reduction in fishing pressure and an improvement in larval recruitment will not lead to rapid increases in lobster stocks, on account of the increased vulnerability of the young lobsters to predators and lack of invertebrate food. The feedback interactions in this system are summarized in Fig. 8.

## Comparison with the Findings of a Workshop on the Subject

The six workshop conclusions of Pringle et al. (1980) were given in the introduction. In places one can find partial support for the hypothesis put forward in this paper, but in general the findings were that data are insufficient to warrant firm conclusions about the interactions between kelp, sea urchins, and lobsters, and no particular course of action by management was called for. Some detailed areas of disagreement between Pringle et al. (1980) and ourselves are discussed below.

Regarding the question of whether sea urchins are the prime cause of kelp disappearance, the following points were mentioned in Pringle et al. (1980, p. 238): the possibility of pollution benefiting the urchins or making the kelp more susceptible to destruction; the possibilities of *Laminaria* being weakened or killed by disease. The difficulty we have in accepting any of these alternatives is that when, after sea urchins had destroyed a kelp bed, an area of 7 × 8 m was cleared of urchins and kept clear for 18 mo, a healthy kelp bed of biomass equal to what had been there before grew up over the whole area within 1½ yr (Breen and Mann 1976a). We should not expect this to happen if the kelp was weakened by pollution or disease.

On the question of whether the populations of urchins have

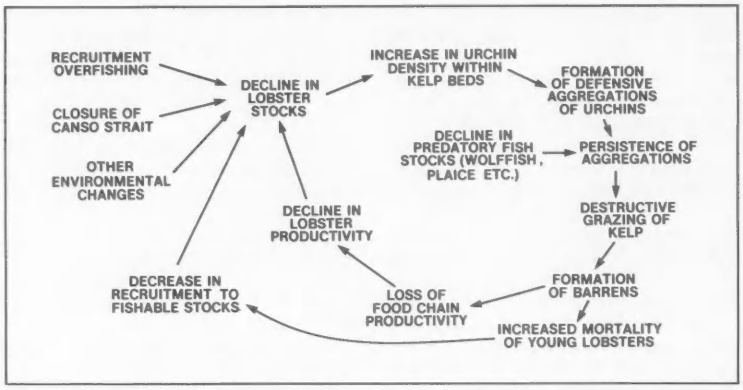


FIG. 8. Diagram summarizing feedback relationship between kelp bed destruction and declining lobster stocks. For details of each interaction, see text of Discussion.

increased in density in large areas of the Nova Scotia coast, we point to the fact that wherever in Nova Scotia dense, stable kelp beds have been investigated, urchin densities have been low, and there is at present an inverse relationship between percentage kelp cover and urchin density (Fig. 2a, b). The workshop accepted the suggestion of a probable decline in kelp biomass and in our view the present evidence favors a parallel increase in urchin abundance.

During the discussion of whether lack of food or lack of plant cover are factors in the decline of lobsters, much of the discussion in Pringle et al. (1980) centered around evidence from work done in the Gulf of St. Lawrence, Newfoundland, and British Columbia. We believe that the ecology of lobsters in other areas is qualitatively different from what we have seen in Nova Scotia. The exposure to open Atlantic swells means a higher proportion of rocky habitats which in their pristine state have dense growths of kelps on their upper surfaces and high population densities of crabs and lobsters in the crevices and spaces between the boulders. In contrast, the Gulf of St. Lawrence has a higher porportion of sedimented shores and British Columbia has no lobsters. Although Pringle et al. (1980) are correct in pointing out that there is insufficient direct evidence to show that kelp removal has led to decreases in lobsters, we maintain that the indirect evidence is strong and is in accord with what we would expect from general ecological considerations. It seems to us highly improbable that the decline in lobster stocks and the disappearance of kelps, in the same general time frame and moving down the coast in the same direction, are entirely coincidental.

Our hypothesis, as now stated, involves the idea that decreases in both crustacean predators and predatory fish are required before an urchin population can destroy kelp beds on a large scale. We are in agreement with those workshop participants who drew attention to the wide variety of predators on urchins. However, the debate about whether lobsters are the prime predators of sea urchins is not now relevant to the issue. We envisage the process as one of positive feedback, so that a decline in productivity of one element begins a general downward spiral in the whole system.

## IMPLICATIONS FOR MANAGEMENT

Two practical problems can be identified: (1) how to halt the spread of destructive grazing from Clark's Harbour west into the prime lobster fishing areas of southwestern Nova Scotia, and (2) how to build up the productivity of the depleted stocks on the Atlantic shore of Nova Scotia.

Dadswell's (1979) theory of recruitment implies that the lobster stocks of southwestern Nova Scotia are unlikely to follow the course of events seen elsewhere because they are subject to a different current pattern and may receive recruits from offshore rather than from places to the northeast along the coast. However, we see (Fig. 7) that destructive grazing has already spread about 100 km into the area that he proposed as a "closed-cell system" and there is room for concern about the future of the lobster stocks within the southwestern cell.

Two views are possible about the situation on the Atlantic coast of Nova Scotia. The first is that kelp bed ecology is irrelevant to the future of the lobster stocks, that the problem is mainly a function of fishing pressure and recruitment, and that the solution lies in control of fishing. The alternative view, which we hold, is that the productivity of the lobster stocks depends strongly on the productivity of the kelp beds and that management strategies should be directed at restoration of kelp as well as of lobster stocks. In California where giant kelp, *Macrocystis*, is harvested in large quantities, there have been localized outbreaks of destructive grazing by *Strongylocentrotus purpuratus* and *S. franciscanus* (North and Pearse 1970) and these have been controlled by the application of quicklime (Wilson et al. 1977). However, the area of kelp rehabilitation has normally been small, of the order of 1 $km^2$ or less, whereas the problem in Nova Scotia extends over hundreds of square kilometres. It would seem to be impracticable and prohibitively expensive to attempt to apply quicklime to the whole area. One possible alternative occurs to us. If urchins could be controlled and kelp restored in restricted areas, and if the density of crustacean predators within them could be brought to the density required to control urchin numbers, those kelp beds might persist for long peri-

ods. If, in addition, stocks of fish predators could be built up to control urchin numbers at the edges of the beds, the kelp might begin to spread from the centers in which it had become established. Clearly, experimental work is needed to determine how many of these possibilities could become practical objectives.

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# Elimination of Hexachlorobenzene (HCB) by Rainbow Trout (*Salmo gairdneri*), and an Examination of its Kinetics in Lake Ontario Salmonids

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NIIMI, A. J., AND C. Y. CHO. 1981. Elimination of hexachlorobenzene (HCB) by rainbow trout (*Salmo gairdneri*), and an examination of its kinetics in Lake Ontario salmonids. Can. J. Fish. Aquat. Sci. 38: 1350–1356.

Subadult rainbow trout (*Salmo gairdneri*) were fed diets containing hexachlorobenzene (HCB) to establish mean body burdens of 225 and 440 μg HCB per fish. They were sampled after 0, 24, 55, and 110 d to estimate the rate of elimination. The biological half-life or $T_{\frac{1}{2}}$ of HCB for trout under the prescribed laboratory conditions was estimated to be at least 7 mo, and perhaps as long as several years. $T_{\frac{1}{2}}$ was calculated using body burden or μg HCB per fish, and tissue concentrations or μg/kg HCB. Estimates of $T_{\frac{1}{2}}$ derived from tissue concentrations ranged from 61 to 117 d, but this was shown to be due to a relative increase in body weight over the study period. The implications of increasing body weight on kinetics measurements are discussed, and an equation is presented that adjusts for changes in body weight where estimates of $T_{\frac{1}{2}}$ are calculated using tissue concentrations.

The results of this and other studies on HCB were used to examine the kinetics of HCB in salmonids collected from Lake Ontario. Using the information derived from laboratory studies on rainbow trout, good agreement was achieved between the expected level and that monitored in field samples of rainbow trout, but only a fair agreement was suggested for lake trout (*Salvelinus namaycush*) and coho salmon (*Oncorhynchus kisutch*). Differences in the HCB kinetics of these species may be attributed to body weight, age, and perhaps feeding habits. Concentrations of HCB reported in field sampling programs generally range from 1 to 100 μg/kg in fish, and it is suggested that these levels are primarily due to the limited availability of HCB in the Lake Ontario environment.

*Key words*: contaminants, hexachlorobenzene (HCB), half-life, elimination, depuration, rainbow trout, *Salmo gairdneri*; Lake Ontario

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On a donné à des truites arc-en-ciel (*Salmo gairdneri*) subadultes des régimes contenant de l'hexachlorobenzène (HCB) de façon à établir des charges corporelles moyennes de 225 et 440 μg de HCB par poisson. Ces derniers ont été échantillonnés après 0, 24, 55 et 110 d afin d'estimer le taux d'élimination. On a estimé à au moins 7 mois, et peut-être même plusieurs années, la demi-vie biologique ou $T_{\frac{1}{2}}$ de HCB chez les truites dans les conditions prescrites de laboratoire. $T_{\frac{1}{2}}$ a été calculé à l'aide de la charge corporelle ou μg de HCB par poisson, et concentrations tissulaires ou μg/kg de HCB. Les estimations de $T_{\frac{1}{2}}$ dérivées des concentrations tissulaires se situent entre 61 et 117 d, mais on a constaté que ceci était dû à une augmentation relative du poids corporel au cours de la période d'étude. Nous examinons les implications de l'augmentation du poids corporel sur les mesures de cinétique, et présentons une équation permettant de faire les ajustements qui tiendront compte des changements de poids corporel quand les estimations de $T_{\frac{1}{2}}$ sont calculées à partir des concentrations tissulaires.

À la lumière de ces résultats et de ceux d'autres études sur HCB, nous avons examiné la cinétique de HCB chez les salmonidés capturés dans le lac Ontario. Faisant appel aux données recueillies à la suite d'études en laboratoire sur la truite arc-en-ciel, nous avons obtenu un bon

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accord entre le niveau anticipé et celui observé dans des échantillons recueillis sur le terrain. Par ailleurs, il y eut seulement accord modéré dans le cas du touladi (*Salvelinus namaycush*) et le saumon coho (*Oncorhynchus kisutch*). Ces différences de cinétique de HCB chez ces espèces peuvent être attribuées au poids corporel, à l'âge et peut-être aux mœurs alimentaires. Les concentrations de HCB notées dans les programmes d'échantillonnage sur le terrain varient généralement de 1 à 100 μg/kg chez les poissons, et nous suggérons que ces niveaux sont causés surtout par la disponibilité limitée de HCB dans l'environnement du lac Ontario.

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HEXACHLOROBENZENE (HCB), $C_6Cl_6$, is an industrial chemical that has been used by the manufacturing sector as a plasticizer for polyvinyl chloride, and a peptizing agent for rubber products (Quinlivan et al. 1975–77; Plimmer and Klingbiel 1976). HCB has also been used as an agricultural fungicide although this practice was restricted in the early 1970's. In view of these applications, there is growing concern about HCB becoming an important environmental contaminant because of its extensive use and its worldwide distribution. HCB concentrations in the fatty tissue of humans average 80 μg/kg in Japan, 40 μg/kg in Italy, 50 μg/kg in the United Kingdom, and 100 μg/kg in Ontario, Canada (Curley et al. 1973; Leoni and D'Arca 1976; Abbott et al. 1972; Holdrinet et al. 1977). Although HCB has not been clearly shown to be a carcinogen, the U.S. Environmental Protection Agency has recommended an interim action guideline of 0.5 mg HCB/kg in animal fat (EPA 1976). It has also been estimated that 3.9 million kg of HCB are indirectly produced annually in the United States as a waste, by-product, or impurity during the manufacture of products such as chlorine, vinyl chloride, and chlorinated solvents (Quinlivan et al. 1975–77). Most of the HCB-containing waste is disposed of in landfills.

HCB is very persistent once it enters the natural environment. No microbial degradation has been observed, photodecomposition is minimal, and volatilization in soil is extremely slow (Verschueren 1977; Plimmer and Kingbiel 1976; Beall 1976). A log *n*-octanol water partition coefficient of 6.18 would indicate that HCB has the potential to bioaccumulate (Neely et al. 1974). Field surveys have reported HCB at ng/kg to mg/kg concentrations in fish collected from different areas in the United States (Johnson et al. 1974). Concentrations of HCB in Lake Ontario salmonids range from 20 to 120 μg/kg (Niimi 1979). HCB has a solubility of 6 μg/L in water although environmental concentrations in waters such as Lake Ontario are in the low ng/L range, and portions of the Mississippi river near industrialized areas may show concentrations in the high ng/L range (Beak 1979; Laska et al. 1976). Laboratory studies have indicated that HCB distribution in a model aquatic community generally corresponds to the trophic levels of the system (Isensee et al. 1976). Studies have also shown that fish fed diets containing HCB attain levels that are related to the concentration in the food and the duration of feeding (Niimi and Cho 1980). This could suggest that fish which occupy the higher trophic levels in an aquatic ecosystem would more likely accumulate HCB through the food chain to a greater degree than direct uptake from water. These observations would be consistent with the behavior of compounds with similar properties such as polychlorinated biphenyls (PCBs) which have been studied in greater detail (Mayer et al. 1977).

To examine the kinetics of HCB in fish and apply the results to a field situation, information on the rate of elimination is required. This rate would establish the net relation between the uptake of HCB and its retention, and clarify the relationship between elimination of HCB and the relative decline of HCB due to growth dilution. The elimination of HCB is established in this study by establishing two levels of body burden in subadult rainbow trout (*Salmo gairdneri*), then monitoring the absolute and relative decline in HCB over several months. Elimination of HCB is then related to earlier studies that examined HCB uptake from food, and HCB levels surveyed in salmonids from Lake Ontario (Niimi and Cho 1980; Niimi 1979).

## Materials and Methods

Hatchery-reared trout were held in 1 × 1 × 0.4 m fiberglass tanks with a capacity of 350 L. Water temperature was kept at 15 ± 1°C and each tank was provided with a 5-L/min flow of dechlorinated domestic water which was passed through an activated charcoal filter. The chemical properties of the water have been described (Hodson 1976). An external pump was used to circulate the water and create a current of 8–12 cm/s to reduce aggressive behavior among fish and facilitate removal of waste products from each self-cleaning tank. The tanks were illuminated by fluorescent lights with a surface intensity of 600 lx which was regulated to a 16-h light: 8-h dark photoperiod.

To obtain fish with similar body burdens of HCB, 29 trout averaging 200 g were placed in each of three tanks and fed on one of three diets to satiation twice daily. The dried pellet feed was formulated according to Cho et al. (1976). One diet contained 7 mg HCB/kg feed which was prepared by dissolving HCB in the salmon oil component of the feed. A second diet, which contained 3 mg HCB/kg feed, was obtained by mixing the first diet and the control feed at a 1:2 ratio. Approximately 4 μg HCB/kg feed was present in the control diet. HCB in the control diet is apparently a result of residual levels of HCB in the ingredients of the fish feed.

After 28 d of feeding on the respective diets, seven fish were sampled from each tank. The remaining fish in all tanks were then fed the control diet on the twice-a-day schedule. Seven fish from each tank were again sampled after 24, 55, and 110 d. All samples were wrapped in aluminum foil and frozen until analysis. One fish fed on the 3-mg HCB/kg diet died of no apparent causes on the 47th day of the experiment. This fish, and the one extra fish from the other two groups,

were not included in the experimental samples.

Fish were analyzed for HCB according to the following procedure. After residual material was removed from the digestive tract, individual fish were weighed and ground with a Hobart grinder and an Oster blender. A sample was prepared for HCB analysis following the method recommended by Environment Canada (1979). During the extraction process, an aliquot of acetonitrile was evaporated to dryness to estimate lipid content. HCB level was determined using a gas chromatograph equipped with an electron capture detector following the conditions described by Niimi and Cho (1980). This method was also used to measure HCB levels in feed. Regression analysis of the relationship between HCB content of the fish and days of elimination followed Snedecor (1961).

## Results

After being fed for 28 d on the respective diets, group A which was fed the 7-mg HCB/kg diet attained a mean concentration of 1654 μg/kg HCB, group B fed the 3-mg HCB/kg diet averaged 964 μg/kg, and group C fed the control diet averaged 5 μg/kg HCB (Table 1). During the following 110 d when all fish were fed the control diet containing 4 μg HCB/kg, there was a significant decrease ($P < 0.01$) in HCB tissue concentrations for groups A and B (Fig. 1). The rate of decline for group B was greater than that of group A; in contrast, HCB levels in group C increased significantly ($P < 0.01$) over the 110-d period.

To compare the elimination of HCB among treatments, half-life ($T_{\frac{1}{2}}$) for each group was determined using the following relationship:

$$\frac{Y_0}{2} = Y_0 e^{-KT_{\frac{1}{2}}}$$

$$T_{\frac{1}{2}} = \frac{-\mathrm{Ln}(\frac{1}{2})}{K}$$

where $Y_0$ is the initial HCB concentration, $K$ the rate constant derived from Fig. 1, and $T_{\frac{1}{2}}$ the time required for the concentration to decrease by 0.5. Using this relationship, the $T_{\frac{1}{2}}$ estimated for groups A and B were 117 and 61 d, respectively. This difference was partially attributed to the greater increase in body weight attained by group B than group A over the

TABLE 1. HCB levels in subadult rainbow trout that were fed 7 mg HCB/kg (group A), 3 mg HCB/kg (group B), and 4 μg HCB/kg (group C) diets for 28 d, then sampled the following 0, 24, 55, and 110 d during which time all fish were fed the 4 μg HCB/kg diet. Values of body weight, lipid, and HCB content represents the mean, standard deviation, and range of seven fish for each treatment.

| | | | HCB level | |
|---|---|---|---|---|
| Days | Body wt (g) mean ± SD range | Lipid (mg/g) mean ± SD range | Concn. (μg/kg) mean ± SD range | Body burden (μg) mean ± SD range |
| | | *Group A* | | |
| 0 | 269±34 | 91±15 | 1654±464 | 437±93 |
| | 248–318 | 79–121 | 1140–2530 | 362–574 |
| 24 | 260±75 | 98±11 | 1651±185 | 421±87 |
| | 200–375 | 84–109 | 1400–1930 | 326–553 |
| 55 | 356±86 | 95±21 | 1077±101 | 378±72 |
| | 245–451 | 66–128 | 980–1280 | 252–446 |
| 110 | 445±110 | 76±8 | 912±187 | 406±116 |
| | 328–677 | 65–83 | 634–1102 | 208–525 |
| | | *Group B* | | |
| 0 | 248±68 | 101±12 | 964±300 | 225±33 |
| | 183–366 | 78–113 | 598–1338 | 182–246 |
| 24 | 360±56 | 91±14 | 642±108 | 227±29 |
| | 273–432 | 76–118 | 503–768 | 194–281 |
| 55 | 533±34 | 101±14 | 279±34 | 149±20 |
| | 470–563 | 81–123 | 237–332 | 129–185 |
| 110 | 626±97 | 88±14 | 274±27 | 173±41 |
| | 583–723 | 69–104 | 248–322 | 147–232 |
| | | *Group C* | | |
| 0 | 252±72 | 95±15 | 5±3 | 1±0 |
| | 180–384 | 75–121 | 3–9 | 1–2 |
| 24 | 278±52 | 88±9 | 9±3 | 2±1 |
| | 229–384 | 78–104 | 6–13 | 1–4 |
| 55 | 380±99 | 88±7 | 10±0 | 4±1 |
| | 273+524 | 74–96 | 10–11 | 3–5 |
| 110 | 489±124 | 84±8 | 12±3 | 6±1 |
| | 323–640 | 76–95 | 9–18 | 4–8 |

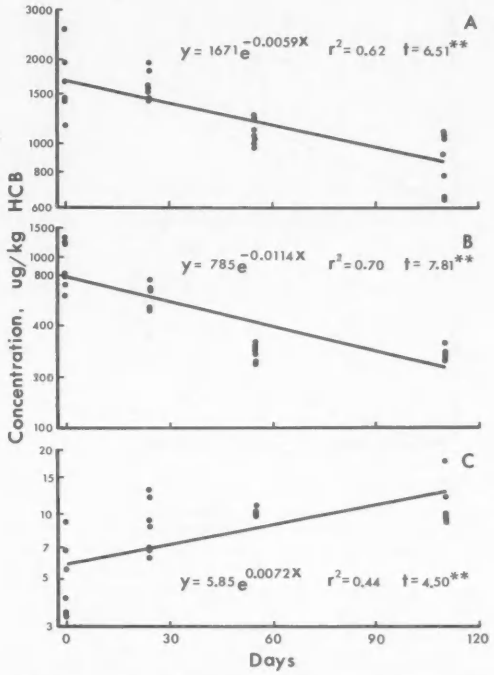


FIG. 1. Changes in concentrations of HCB, expressed as μg/kg, in subadult rainbow trout that had attained mean concentrations of 1650 (A), 960 (B), and 5 (C) μg/kg HCB. Fish were fed a prepared diet containing 4 μg HCB/kg after they had attained these levels during the following 110 d. Each equation was calculated from a sample of 28 fish. All equations are statistically significant at the $P < 0.01$ level.

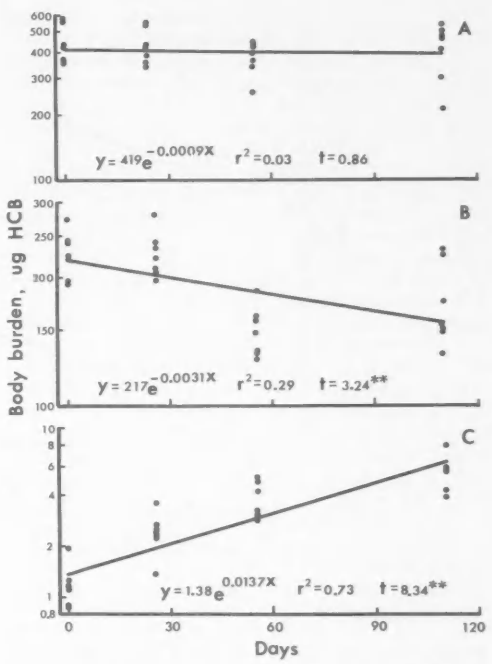


FIG. 2. Changes of body burden of HCB, expressed as μg HCB per fish, in subadult rainbow trout that had attained mean concentration of 440 (A), 225 (B), and 1 (C) μg of HCB. Each equation was calculated from a sample of 28 fish. Equations B and C are statistically significant at the $P < 0.01$ level.

110-d period. To minimize the influence of weight, elimination of HCB was based on changes in body burden or μg HCB per fish. HCB body burdens after the 28-d-exposure period averaged 437, 225, and 1 μg per fish for the respective groups (Table 1). Group A showed no significant decrease ($P > 0.05$) while group *B* showed a significant decrease ($P < 0.01$) in HCB content over 110 d (Fig. 2). Using the estimated slopes based on the decrease in body burden, $T_{\frac{1}{2}}$ was estimated as 224 d for group B, and 770 d for group A which suggested no significant changes in HCB levels over 110 d.

No adjustment was made for the intake of HCB from the control diet over the 110-d clearance period. Based on a feed-to-gain ratio of 1.3:1, HCB uptake from food would be less than 2 μg for all groups even after 110 d.

## Discussion

The half-life of HCB was calculated using tissue and body burden levels, and the results were different. In retrospect, this apparent difference is attributed to the method of measurement, and the intended application of each estimate. $T_{\frac{1}{2}}$ can be used to measure changes in the level of a compound in an organism, and most estimates are derived following one of two procedures. Estimates based on muscle samples could have two applications. $T_{\frac{1}{2}}$ may be used to estimate the time required for an environmental contaminant to attain a certain concentration in the edible portion of fish as viewed from a human health perspective. Muscle samples may also be used to examine the kinetics of a compound although this practice may be influenced by several undesirable factors. Muscle biomass increases proportionately faster than other components of the proximate body composition; this is particularly evident in young fish.

When measurements are made on samples derived from whole animal homogenates, two estimates of $T_{\frac{1}{2}}$ are possible as demonstrated in this study. The estimates derived would be in good agreement only if the change in body weight over the duration of the study was negligible. Any increase in body weight during the period of elimination would proportionately reduce the concentration.

Estimates of $T_{\frac{1}{2}}$ based on whole fish homogenates and calculated from concentrations can be adjusted to actual $T_{\frac{1}{2}}$ values if the growth rate of the organism is known. Following the general concept of Pentreath and Jefferies (1971), the growth constant λ can be incorporated into the $T_{\frac{1}{2}}$ relationship to account for changes in body weight, provided growth is an

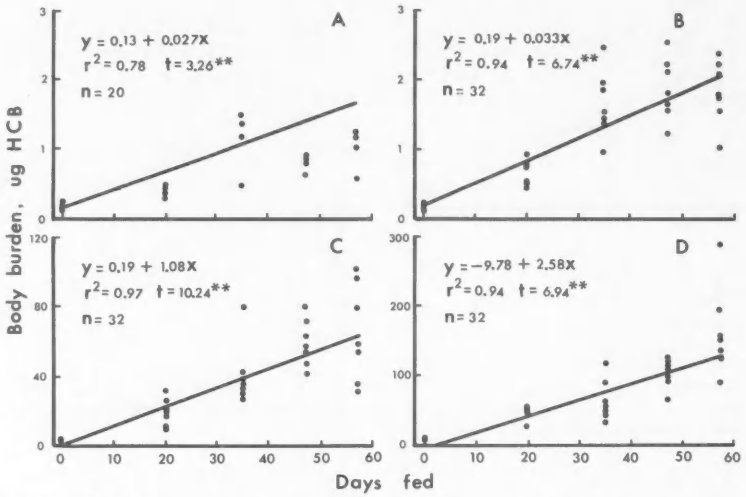


FIG. 3. Relationship between body burden of HCB and duration of feeding in 100−240 g rainbow trout fed to satiation twice daily on diets containing 6 (A), 8 (B), 394 (C), and 780 (D) μg HCB/kg over 57 d (modified after Niimi and Cho 1980). All equations are statistically significant at the $P < 0.01$ level.

exponential function. To estimate $T_{\frac{1}{2}}$ using concentrations, the following relationship can be applied:

$$T_{\frac{1}{2}} = \frac{-\mathrm{Ln}(\frac{1}{2})}{K + \lambda}$$

where $K$ is the rate constant for the decrease in concentration, and $\lambda$ the rate constant of growth of the organism. An application of this relationship to the results of this study provided good agreement. When the growth constant $\lambda$, estimated as 0.0082 from Table 1, was applied to the decline in the HCB concentration for whole fish in group B (Fig. 1), $T_{\frac{1}{2}}$ was estimated as 217 d. This value is in agreement with the $T_{\frac{1}{2}}$ of 224 d derived from body burden measurements (Fig. 2), and is in marked contrast to the 61-d estimate based on the decrease in concentration. A similar application to group A suggested a $T_{\frac{1}{2}}$ of 866 d, although the initial estimate of 770 d derived from body burden was not significantly related to time.

Under the prescribed experimental conditions, the $T_{\frac{1}{2}}$ calculated for HCB in subadult rainbow trout would be in excess of 7 mo and may be as long as several years. This estimate is considerably longer than those reported for fish in other studies. Neely et al. (1974) estimated a clearance rate ($K$) of 0.00238/h, or a $T_{\frac{1}{2}}$ that would be equivalent to 12 d in rainbow trout, but the study was based on muscle samples of 8−10 g fish fed 3× daily for an unspecified period. Sanborn et al. (1977) reported an apparent $T_{\frac{1}{2}}$ of 8−20 d in different tissues of green sunfish (*Lepomis cyanellus*) that was presumably determined on young fish maintained up to 28 d; $T_{\frac{1}{2}}$ was estimated using tissue concentrations. Because growth dilution was not accounted for in these studies, it would appear that the actual $T_{\frac{1}{2}}$ for HCB could be longer than those reported, perhaps in the order of several months or more than several weeks. In homeotherms, $T_{\frac{1}{2}}$ values of 2−4 mo for rats and 2.5−3 yr for monkeys have been reported for HCB (Morita and Oishi 1975; Muller et al. 1978).

Laboratory studies suggest ~80−90% of HCB ingested in food is retained by trout fed diets containing 6−780 μg HCB/kg (Niimi and Cho 1980). At these high retention rates, the concentration of HCB attained can be strongly influenced by the level of HCB in the food and the duration of feeding (Fig. 3). Estimates of HCB from a natural population of fish would also suggest a relatively long half-life for HCB. Lake trout (*Salvelinus namaycush*), rainbow trout, and coho salmon (*Oncorhynchus kisutch*) from Lake Ontario had average HCB concentrations of 80, 62, and 36 μg/kg, equivalent to body burdens of 82, 144, and 128 μg HCB, for the respective species (Niimi 1979). The relationship between body burden and weight was quite distinct for each species which suggests differences in their physiology and/or feeding habits (Fig. 4). Interspecific differences are suggested when the growth requirements of a 3-kg fish are examined. Using a gross conversion efficiency for food of 0.3, a piscivorous species would require ~9 kg of food to attain 3 kg in weight (Niimi and Beamish 1974). Rainbow trout and coho salmon in Lake Ontario forage predominantly on rainbow smelt (*Osmerus mordax*), and alewives (*Alosa pseudoharengus*) which can average 24 μg HCB/kg (Norstrom et al. 1978). Total HCB intake from food for the 3-kg fish would be about 220 μg of which ~190 μg would be retained based on an 85% retention efficiency.

Direct uptake of HCB from water can be a contributing factor in the kinetics of a substance. Laboratory experiments have demonstrated that fish exposed to waterborne HCB at concentrations of 3−8 μg/L for 20−120 d can bioaccumulate HCB 400−18500× (Isensee et al. 1976; Veith et al. 1979; Konemann and van Leeuwen 1980). Most of the uptake

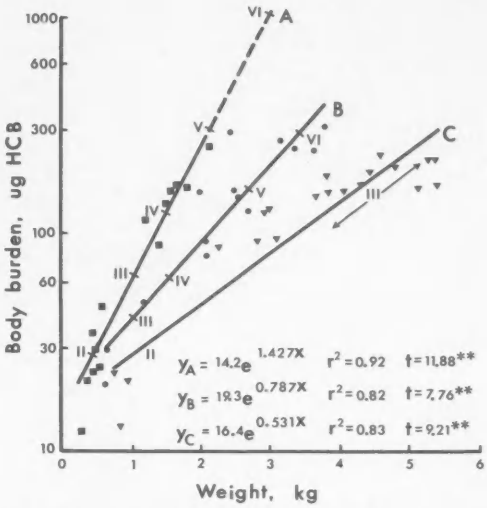


FIG. 4. Relationship between body burden of HCB and body weight in lake trout (A), rainbow trout (B), and coho salmon (C) collected from Lake Ontario (modified after Niimi 1979). The roman numerals represent fish age based on the mean weight at each age-class estimated for lake trout from eastern Lake Ontario sampled between 1975 and 1979 (W. J. Christie, Ministry of Natural Resources, Picton, Ont., personal communication) and the lake-run rainbow trout from the Ganaraska River which were stream residents for 2 years sampled between 1974 and 1979 (R. M. Biette, Ministry of Natural Resources, Toronto, Ont., personal communication). All equations are statistically significant at the $P < 0.01$ level.

occurs during the first 10 d. While uptake from water can be a significant factor in some aquatic systems with elevated HCB levels in the water, it is suggested that uptake from water is not a major source of HCB for Lake Ontario salmonids. HCB measurements of Lake Ontario waters indicate levels in the quadrillion (pg/L) range. Bulk water samples taken off Port Dalhousie indicated no detectable levels (theoretical detection limit of 0.5 ng/L) of HCB (J. D. Fitzsimons, Canada Centre for Inland Waters, Burlington, Ont., personal communication). Offshore sampling stations indicate HCB levels of 0.1 ng/L or less (B. G. Oliver, Canada Centre for Inland Waters, Burlington, Ont., personal communication), while water samples from the Niagara River area contain 0.1–0.2 ng/L HCB (Oliver and Bothen 1980). In view of these concentrations, uptake of HCB from water by top carnivores in the Lake Ontario environment is suggested to be considerably less than that obtained from food. Similar views have been suggested for other persistent contaminants such as DDT and dieldrin where concentrations approaching Great Lakes environmental levels have been examined (Macek and Korn 1970; Reinert 1972; Thomann 1981). Any uptake of HCB from water would increase the estimated 190 μg HCB retained from food by rainbow trout. With a prolonged $T_{\frac{1}{2}}$, this estimate would be in good agreement with the levels monitored in rainbow trout from Lake Ontario (Fig. 4). The differences with lake trout and coho salmon may be attributed to physiological and ecological differences.

Body weight and lipid levels are factors that could markedly affect the kinetics of a compound. Norstrom et al. (1976) demonstrated the effect of increasing body weight on decreasing elimination rate on the kinetics of PCB and methylmercury in yellow perch (*Perca flavescens*). Body weight of fish in the present study represented only a small portion of its range which was not sufficient to examine the relationship between $T_{\frac{1}{2}}$ and body weight. Lipid levels could also affect the kinetics of a lipophilic organic contaminant where retention has been shown to be directly related to lipid content (Roberts et al. 1977). Lipid concentrations in fish in the present study, which averaged 76–101 mg/g (Table 1), are similar to the 89-mg/g average monitored in rainbow trout from Lake Ontario (Niimi 1979). Lipid levels in lake trout and coho salmon averaged 162 and 75 mg/g, respectively.

The differences among rainbow trout, lake trout, and coho salmon may be attributed to other factors. The lake trout monitored included 2 to 5 yr-old fish, rainbow trout perhaps 3–6 yr old, and coho salmon 2–3 yr old. Both age and weight appear to influence kinetics. HCB levels increase with body weight among the three species, and in addition, lake trout contain higher levels of HCB than rainbow trout of the same age, although the body weight of the rainbow trout may be equal or greater (Fig. 4). A similar comparison cannot be made for the rapidly growing, short-lived coho salmon. Where age becomes an important factor, uptake from water could be a significant pathway, particularly for a long-lived species such as lake trout. Differences in feeding habits could also influence HCB levels. In Lake Ontario, it is suggested that lake trout feed on sculpins, which feed on the benthic dwelling *Pontoporeia*. Rainbow trout and coho salmon prey on alewives and smelt which feed upon *Mysis* and other microcrustaceans that inhabit the water column.

Overall, samples of fish taken from different waters in North America suggests most species contain HCB in the low μg/kg range (Johnson et al. 1974; Frank et al. 1978; Niimi 1979). Laboratory studies have demonstrated that concentrations of HCB in the mg/kg range can be readily attained if HCB is present in similar concentrations in the food (Niimi and Cho 1980). However, this does not appear to be the case for HCB in fish from most natural water bodies. It is suggested that the levels of HCB monitored in fish from natural waters is attributed to the limited availability of HCB in the aquatic environment. It is further suggested that in some systems such as Lake Ontario where waterborne levels of HCB are extremely low, uptake from food would be substantially greater than that from water.

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# Developmental Responses of Juvenile *Daphnia rosea* to Experimental Alteration of Temperature and Natural Seston Concentration

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A need for accurate estimates of juvenile development rates of *Daphnia rosea* from oligotrophic lakes prompted this investigation into the combined effects of temperature and natural seston concentrations on growth of neonates. Significant effects of seston concentration and temperature and especially their interaction were detected upon survival, number of prereproductive instars, growth increment per instar, and duration of development. Low summertime seston concentrations that were sufficient for good survival and growth at a low rearing temperature (12°C) generally produced high mortality, slow development, and reduced biomass increments at higher temperature (21°C). Further, poor growth and survival under low seston–high temperature conditions were exaggerated in August compared with May, suggesting possible seasonal changes in natural seston quality and/or juvenile daphnid physiology. In contrast, higher food levels typical of springtime conditions produced excellent survival and growth regardless of the test temperature employed or season examined. Food limitation of daphnid development was thus dependent upon both temperature and food concentration within the range of conditions commonly observed in oligotrophic lakes. Extrapolation of development rates measured under only a limited range of food–temperature conditions or taken from literature values should be done cautiously.

*Key words*: *Daphnia*, development, juvenile, seston, food, temperature, growth, survival, Cladocera, oligotrophic

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L'étude décrite dans l'article qui suit sur les effets combinés de la température et des concentrations de seston naturel sur la croissance des nouveaux-nés a été dictée par le besoin d'estimations précises des taux de développement de jeunes *Daphnia rosea* habitant des lacs oligotrophes. On a détecté des effets significatifs de la concentration du seston et de la température, et surtout de leur interaction, sur la survie, le nombre de larves prépreproductrices, la croissance par stade larvaire et la durée du développement. Les faibles concentrations estivales de seston qui suffisent à une bonne survie et croissance à une basse température d'élevage (12°C) causent généralement de fortes mortalités, un lent développement et un accroissement réduit de biomasse à une température plus élevée (21°C). En outre, une faible croissance et survie dans des conditions de faible abondance du seston et de haute température s'accentuent en août par rapport à mai, ce qui laisse supposer l'existence de changements saisonniers possibles dans la qualité du seston naturel et (ou) dans la physiologie des jeunes daphnies. Par contre, les hauts niveaux de nourriture typiques des conditions printannières produisent une excellente survie et croissance quelle que soit la température d'essai ou la saison. La limitation du développement des daphnies par la nourriture dépend donc à la fois de la température et de la concentration de la nourriture dans l'éventail de conditions communément observées dans les lacs oligotrophes. On devrait donc être prudent dans l'extrapolation des taux de développement mesurés seulement sur un éventail limité de conditions de nourriture et de température, ou extraits des travaux publiés.

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ACCURATE estimates of the rates of juvenile development are important in studies of zooplankton population dynamics and productivity. As in all poikilotherms, zooplankton development rates are highly temperature dependent, frequently varying as a "normal" Krogh curve, provided that no other factors are limiting (Edmondson 1971). Food limitation of development has seldom been investigated and most authors have been content to restrict their interest to the effects of temperature (e.g. Hall 1964; Hrbackova-Esslova 1966; Bottrell 1975; Landry 1978; and many others). In their reviews of the cladoceran literature, Green (1956) and Hebert (1978) concluded that temperature is virtually the only factor affecting juvenile development rates in nature, although both authors recognized possible food effects in laboratory experiments. Although all of his data were taken from food-rich laboratory cultures, Lynch (1980) recognized the importance of food limitation to his models of cladoceran life histories. Kryuchka's (1973) study of several cladoceran species and McLaren's (1978) analysis of growth in seven species of copepods from Loch Striven are good examples of this temperature-centered view.

Weglenska (1971) clearly showed that experimental alteration of natural seston levels had major effects upon the development rates and sizes of both cladocerans and copepods in a eutrophic lake. Similar to the effect of temperature on development time, the effect of increasing food supply was curvilinear, with its largest impact occurring at low seston concentrations. Weglenska (1971) assumed, however, that altering temperature away from a standard 18°C would merely scale these food effects up or down without affecting the mathematical form of the relationship. She did not consider the potential interaction of temperature and food supply such that a nonlimiting concentration of food at one temperature could limit growth at a higher temperature. Particularly if food quality was poor, harvest rates or assimilation rates of required nutrients, for example, might lag behind qualitatively and quantitatively altered metabolic demands at higher temperatures (Blazka 1966).

As part of a study on dynamics of the crustacean zooplankton species in oligotrophic lakes in coastal British Columbia, I examined development rates of juvenile *Daphnia rosea* under representative conditions of temperature and natural food. The purpose of this paper is to report evidence of a strong food–temperature interaction in development of this species.

## Methods

Ovigerous *Daphnia rosea* were collected by tow net during May and August 1976 in Placid Lake, a small (2.5 ha), shallow (maximum depth 6 m), but well stratified oligotrophic lake in the University of British Columbia Research Forest, near Haney, B.C., Canada, 60 km east of Vancouver. General limnological features of this lake and others in the Forest have been described by Efford (1967), Northcote and Clarotto (1975), and Neill (1978). Lake water was collected daily from a depth of 1.5 m (depth of maximal juvenile *D. rosea* density), passed through a 73-μm nylon seive to remove crustaceans, most rotifers, and debris, and transported to the laboratory in an ice bath. Fresh neonate *Daphnia* (from adults collected within 24 h) were introduced individually into 200-mL jars containing lake water at three concentrations of natural seston: $0.4 \pm 0.05$ mg·L$^{-1}$, $0.7 \pm 0.05$ mg·L$^{-1}$, and $1.0 \pm 0.05$ mg·L$^{-1}$ dry weight. Suspensions of natural seston were prepared daily by gently concentrating seston through a 0.45-μm filter in a device modified from the design of Morris and Yentsch (1972) or by diluting seston with 0.45-μm-filtered lake water. Seston suspensions in all growth vessels were gently agitated approximately midway through each 24-h period to minimize settling of particulates. Lake water seston concentration varied seasonally in 1976. In spring, seston levels were 0.9–1.0 mg·L$^{-1}$ whereas after midsummer they were only 0.3–0.7 mg·L$^{-1}$ dry weight. Microscopical examination of fresh seston and concentrated/diluted natural seston revealed only a 5–15% reduction in the proportion of phytoflagellates (mainly cryptomonads) after concentration. No statistically significant changes were detected in the proportions of chlorophytes (*Chlorella, Oocystis, Scenedesmus*), coccoid blue-greens (*Synechocystis*, others?), chrysophytes (*Mallomonas, Ochromonas*), diatoms (*Navicula, Fragilaria, Synedra*), or detritus (which appeared to comprise 75–95% of the seston biomass by volume).

Jars containing the three prepared media and juvenile *Daphnia* were incubated at constant 12, 15, or 21°C under a fluorescent light cycle of 16 h light:8 h dark. The animals were measured microscopically daily and freshly prepared medium was placed in all jars. Control jars for evaluating effects of handling, in which juveniles were transferred daily to new medium but were examined microscopically only every 4 d, were set up as well. Although there was a trend for control animals to be slightly larger than those handled daily, the difference was not significant statistically ($F$-test, $P > 0.05$). There was no trend in length of the prereproductive period, and so I concluded that handling had no significant influence upon juvenile development in these studies.

## Results

Experimentally manipulating temperature and concentration of natural seston altered both survival and the number of prereproductive instars of *D. rosea*. Table 1 summarizes these data for juveniles from Placid Lake in May and August 1976. Juvenile survival was uniformly high at all test temperatures in both May and August when the concentration of natural seston was kept artificially high. Survival was also good at lower seston concentrations whenever test temperatures were kept relatively cool (12 or 15°C). Note, however, that at 15°C, juvenile survival in August was only half that in May at the lowest seston concentration. In contrast with these results, survival under conditions of low seston concentration and high temperature was uniformly poor. Further, comparing May versus August survival in the 21°C treatments reveals that juveniles in spring generally were more successful than those in summer ($G$-test, $P < 0.05$). In sum, at epilimnetic temperatures typical of spring (≤15°C), there were no important nutritional or seasonal effects on juvenile survivorship. At typical summertime temperatures (>20°C), however, animals survived much better when test seston was present in relatively high concentrations or came from spring collections. Thus, a clear temperature–food

TABLE 1. Proportion of initial neonate number ($n$ = 40) that matured at each instar under different combinations of temperature and seston concentrations. Proportions summing to less than 1.0 indicate prereproductive mortality.

| Temp. | Primaparous instar | Seston concentration: 0.4 mg·L$^{-1}$ May | 0.4 mg·L$^{-1}$ Aug. | 0.7 mg·L$^{-1}$ May | 0.7 mg·L$^{-1}$ Aug. | 1.0 mg·L$^{-1}$ May | 1.0 mg·L$^{-1}$ Aug. |
|---|---|---|---|---|---|---|---|
| 12°C | IV | 0 | 0 | 0.25 | 0.10 | 0.38 | 0.15 |
| | V | 1.00 | 1.00 | 0.75 | 0.90 | 0.62 | 0.85 |
| | VI | 0 | 0 | 0 | 0 | 0 | 0 |
| | VII | 0 | 0 | 0 | 0 | 0 | 0 |
| 15°C | IV | 0 | 0 | 0 | 0 | 0.28 | 0.05 |
| | V | 1.00 | 0.50 | 1.00 | 1.00 | 0.72 | 0.95 |
| | VI | 0 | 0 | 0 | 0 | 0 | 0 |
| | VII | 0 | 0 | 0 | 0 | 0 | 0 |
| 21°C | IV | 0 | 0 | 0 | 0 | 0.15 | 0 |
| | V | 0.13 | 0 | 0.35 | 0.15 | 0.85 | 1.00 |
| | VI | 0.08 | 0 | 0.10 | 0.08 | 0 | 0 |
| | VII | 0 | 0 | 0 | 0.08 | 0 | 0 |

TABLE 2. Mean (± 1 SD) body sizes (in mm) of primaparous *D. rosea* when reared under different seston and temperature conditions.

| Temp. | 0.4 mg·L$^{-1}$ May | 0.4 mg·L$^{-1}$ Aug. | 0.7 mg·L$^{-1}$ May | 0.7 mg·L$^{-1}$ Aug. | 1.0 mg·L$^{-1}$ May | 1.0 mg·L$^{-1}$ Aug. |
|---|---|---|---|---|---|---|
| 12°C | 1.18±0.33 | 1.23±0.26 | 1.19±0.29 | 1.20±0.30 | 1.24±0.36 | 1.17±0.36 |
| 15°C | 1.16±0.24 | 1.14±0.29 | 1.14±0.26 | 1.16±0.30 | 1.20±0.24 | 1.11±0.31 |
| 21°C | 1.06±0.27 | — | 1.04±0.21 | 1.07±0.33 | 1.07±0.24 | 1.03±0.21 |

concentration interaction existed with respect to juvenile survival.

To characterize the differences in growth patterns under these conditions, the number of prereproductive instars among *Daphnia* in all treatments is also shown in Table 1. Food concentration and temperature interacted significantly here as well. In general, juveniles reached reproductive maturity in fewer instars when seston was abundant and temperatures low than when seston was scarce or temperatures elevated. For example, compared with the 12°C and 1.0 mg·L$^{-1}$ treatment, as many as three additional prereproductive instars were produced by the few surviving juveniles in the high temperature−medium seston treatment. Food conditions were so poor in the high temperature−low seston treatment that no juveniles survived to maturity. Temperature itself had only small effects on numbers of instars so long as food concentration was high (chi-square test, $P < 0.05$), but at lower food densities additional instars were inserted before individuals became reproductively active at 21°C vs. 12°C (chi-square test, $P < 0.001$).

Within seston treatments in both May and August, temperature was negatively associated with body length at the first reproductive instar ($F$-test, $P < 0.05$). Hence, as is commonly observed in temperature studies of poikilotherm development, smaller primaparous adults were produced under warmer temperatures (Table 2). Within temperature treatments, however, there were no detectable effects of seston concentration upon mean body size at the primaparous instar (Table 2, $P > 0.05$). This was a surprising result as clear effects of seston concentration upon growth increment per juvenile instar had been observed (Table 3). Once again, the effects were not simple but varied with temperature. At cool temperatures, growth increment was large and uniform across food concentrations. At the highest temperature, however, growth increment per instar was reduced, but to a much greater degree at low food levels than high. Juveniles at high temperatures and low food grew very little. Nevertheless, differences between mean sizes of primaparous individuals at a temperature were minimal. Apparently, the insertion of additional prereproductive instars under conditions of low seston concentration compensated for reduced growth per juvenile instar. During adult instars, continued low growth per instar, especially under extreme conditions of low food and high temperature (Table 3), resulted in smaller adults than under more abundant food conditions. Disproportionately

TABLE 3. Growth increment (in mm) ± 1 SD of *D. rosea* in molt between instars III and IV and instars VII and VIII under different seston and temperature conditions. Data are for August 1976 only.

| Temp. | Molt | 0.4 mg·L$^{-1}$ | 0.7 mg·L$^{-1}$ | 1.0 mg·L$^{-1}$ |
|---|---|---|---|---|
| 12°C | III−IV | 0.21±0.06 | 0.20±0.07 | 0.24±0.07 |
| | VII−VIII | 0.07±0.02 | 0.07±0.02 | 0.10±0.03 |
| 15°C | III−IV | 0.13±0.05 | 0.20±0.05 | 0.26±0.08 |
| | VII−VIII | 0.03±0.01 | 0.07±0.02 | 0.10±0.02 |
| 21°C | III−IV | 0.02 | 0.05±0.02 | 0.12±0.03 |
| | VII−VIII | — | 0.04±0.02 | 0.09±0.02 |

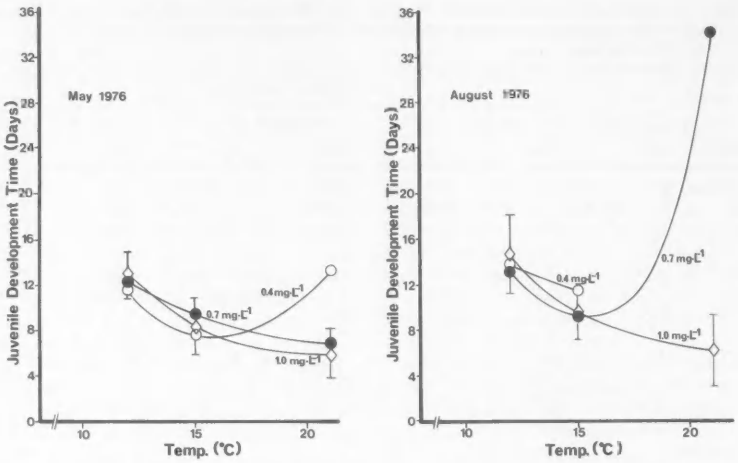


FIG. 1. Duration of juvenile development of *D. rosea* under various combinations of temperature and concentration of seston collected in May and August 1976. Vertical bars represent 95% confidence intervals for 1.0 mg·L$^{-1}$ seston treatments.

greater suppression of adult growth occurred under summertime food and temperature conditions than under springtime conditions in Placid Lake.

Figure 1 summarizes the data on duration of the prereproductive period of juvenile *D. rosea* under the experimental conditions employed. No precise data are available on the duration of each instar as individuals were only examined once daily and some instar durations were only 20–30 h. There was a clear curvilinear relation between length of prereproductive period and temperature (a typical Krogh curve) at 1.0 mg·L$^{-1}$ seston. In addition, the curves from spring versus summer studies were nearly congruent at this food level. At seston concentrations lower than 1.0 mg·L$^{-1}$, the duration of juvenile development at higher temperatures increased rather than decreased as expected. In particular, in May 1976, animals reared in 0.4 mg·L$^{-1}$ seston required twice as long to reach maturity at 21°C as those reared at 0.7 mg·L$^{-1}$ and 1.0 mg·L$^{-1}$ at that temperature. In contrast, at lower temperatures, there were no differences in duration of the juvenile period associated with seston density. All levels of food were apparently sufficient for normal development at low temperatures but not at high temperatures. An even stronger food–temperature interaction occurred in August 1976: juveniles in 0.7 mg·L$^{-1}$ seston at 21°C required six times as long to mature as those held at 1.0 mg·L$^{-1}$ at the same temperature. Further, in contrast with results from May 1976, no animals even survived to reach maturity in August at 21°C and 0.4 mg·L$^{-1}$, the last individual dying while molting from instar 3 to 4 on day 55 of the experiment. This molt had been completed ~4 wk earlier in juveniles at 0.7 mg·L$^{-1}$ and 21°C and 6 wk earlier in those at 1.0 mg·L$^{-1}$ and 21°C . Thus, although similar seston concentrations were provided in May and August, juvenile development was much slower in seston collected in August. Natural seston levels in Placid Lake in August 1976 were only 0.4–0.7 mg·L$^{-1}$ and very few juvenile *Daphnia* were observed in routine samples.

To assess whether changes in seston quality may have occurred by August, microscopic enumeration and volume estimation were used to try to characterize the seston in these experiments. Total seston weight was estimated gravimetrically after combustion at 600°C. The average total amount of "grazable" algae (particles <30 μm) in unmanipulated lake water appears to have been larger in spring (0.217 mg·L$^{-1}$ vs. 0.127 mg·L$^{-1}$ dry weight for May and August, respectively) while undefinable detrital material predominated (0.763 mg·L$^{-1}$ vs. 0.453 mg·L$^{-1}$ for May and August, respectively). Hence, although the amounts of both algae and total seston in the lake were greater in spring, the ratio of algae to seston (about 0.22) fortuitously did not differ from May to August experiments. Nevertheless, the taxonomic composition of the algae changed markedly during summer and may have influenced the nutritional value of the seston to young daphnids. In spring of 1976, "blooms" of diatoms (especially *Fragilaria* and *Navicula*) and various monads (including *Ochromonas, Cryptomonas, Chilomonas*, and others) predominated. In summer, however, these forms were extremely rare and the algae were dominated by *Scenedesmus* and the coccoid blue-green alga *Synechocystis*. Qualitative changes in the detrital component of the seston could not be characterized.

## Discussion

Accurate estimates of juvenile development rates of zooplankton are central to a wide variety of theoretical and applied problems in aquatic ecosystems. For example, estimates of the efficiency of trophic transfer, instantaneous population growth rates, impact of predators upon prey populations, or stocking rates of planktivorous fish species may depend critically upon knowledge of juvenile development. Lynch (1980) lamented the scarcity of field data on juvenile development and survival in his analysis of cladoceran life

histories.

By altering concentrations of natural seston in the laboratory, Weglenska (1971) showed that, even in eutrophic lakes, juvenile development rates could be slowed by limited food supplies. In less productive waters, limited food supplies may be all the more important. My results from a factorial experiment using natural seston show that, in addition to direct effects of food limitation and temperature on development, these two factors may interact strongly. Under experimental conditions of low food and warm temperature, which replicate midsummer conditions, survival was very poor, additional instars were inserted in development, growth per instar was retarded, and duration of the juvenile period was protracted compared with those reared under less limiting food conditions. As temperature increases, metabolic needs for some nutrient material(s) apparently increase faster than the abilities of juvenile organisms to obtain them when food concentrations are low. Merely scaling up food effects or temperature effects that had been determined under more salutory conditions would have falsely predicted developmental behavior under more stressful summertime conditions. That higher test temperatures alone were not responsible for poor juvenile performance is clear from the excellent growth of animals under high temperature–high seston conditions.

Further, the developmental behavior of juvenile *D. rosea* from Placid Lake was not constant across seasons. Exposure of neonates to low concentrations of springtime seston produced markedly better development than summer seston under warm test conditions. Whether nutritional, genetical, or physiological changes produced the differences in responses of juveniles in May versus August is unclear. Substantial seasonal changes in the types and abundances of phytoplankton certainly occurred in Placid Lake in 1976. The seasonal decline in abundance of monads, diatoms, and bacterioplankton, coupled with an increasing density of poorly digestable *Synechocystis* (Arnold 1971) and perhaps more refractory detritus (Saunders 1969), may have differentially affected juvenile development. For routine studies in the absence of other information, it seems prudent to consider the use of seston which has been permitted to vary naturally in nutritional value and to repeat developmental studies frequently near the extremes of environmental variation. Although tedious, in situ life history studies may be the most effective way to account directly for hidden interaction effects (Threlkeld 1979).

Depth-stratified Clark-Bumpus sampling along the entire length of Placid Lake reveals that, unlike adults, juveniles of all crustacean species are found almost exclusively in the upper epilimnion. They do not migrate vertically. Temperatures at such shallow depth typically exceed 20°C during July and August. Slow juvenile development and substantial starvation-related mortality are thus likely during these months. Strong selection pressures, perhaps predation from *Cyclops bicuspidatus thomasi*, may preclude descent to deeper depths which would otherwise be expected to produce higher developmental success. Alternatively, the development rates measured in jars in the laboratory may have exaggerated in situ conditions, despite the use of natural seston and lake water. The reduction of turbulent mixing (suspensions were gently stirred once daily) and the absence of temporal patchiness in food abundance or short-term temperature fluctuations may have produced less favorable growth conditions in jars. Unfortunately, confinement of individuals is unavoidable in such studies and the time scales at which food–temperature heterogeneities may normally be encountered in situ are unknown. However, the magnitude of the food–temperature interaction detected under low seston and high temperature conditions (Fig. 1) suggests that subtle differences between conditions in the lake and in jars are unlikely to account for the response.

In sum, significant biases can apparently occur in extrapolating development rates of juvenile daphnids of oligotrophic waters from studies using only limited combinations of food quantity–quality and temperature. Food limitation of development can occur at elevated temperatures when these food levels have been sufficient at lower temperatures. Caution should be exercised in employing literature values without experimentation in calculating estimates of zooplankton population parameters and productivity.

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# Predictive Models of Benthic Macroinvertebrate Density for Use in Instream Flow Studies and Regulated Flow Management

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GORE, J. A., AND R. D. JUDY JR. 1981. Predictive models of benthic macroinvertebrate density for use in instream flow studies and regulated flow management. Can. J. Fish. Aquat. Sci. 38: 1363 – 1370.

As an adjunct to the U.S. Fish and Wildlife Service incremental method for determining habitat suitability for sport fish species at various discharge levels, we propose a similar method for determining optimum conditions for stream macroinvertebrates that stresses predictions of density maxima and flow predictions for maintenance of suitable lotic habitat.

The curve fitting equations of the computer simulation showed high reliability in predicting physical habitat tolerances of selected aquatic insects. An example using *Nectopsyche lahontonensis* is presented.

The new predictive model, by exponential polynomial analysis, answers criticisms of earlier models which do not include the dependence of velocity and depth in stream channel hydraulics. The added cross-product term in the model altered the predicted peak responses (density) with random changes in depth and velocity. Although the new model improves the predictions, its effectiveness in stream management has yet to be tested.

*Key words*: habitat suitability, macroinvertebrates, exponential polynomial analysis, flow management, *Nectopsyche lahontonensis*

GORE, J. A., AND R. D. JUDY JR. 1981. Predictive models of benthic macroinvertebrate density for use in instream flow studies and regulated flow management. Can. J. Fish. Aquat. Sci. 38: 1363 – 1370.

L'article qui suit contient la description d'une méthode de détermination des conditions optimales pour les macro-invertébrés de cours d'eau, dans laquelle on met l'accent sur les prévisions de maxima de densité et de débit d'eau pour le maintien d'un habitat lotique convenable. Cette méthode fait pendant et est semblable à la méthode par accroissements du *U.S. Fish and Wildlife Service* utilisée pour déterminer si l'habitat, à divers débits, convient à certaines espèces de poissons de sport.

Les équations correspondant aux courbes obtenues par simulation machine démontrent qu'il est possible de prédire, avec un haut degré de fiabilité, les tolérances au milieu physique d'insectes aquatiques choisis. Nous donnons un exemple tiré de *Nectopsyche lahontonensis*.

Par le biais de l'analyse polynomiale exponentielle, le nouveau modèle prévisionnel permet de réfuter les critiques de modèles antérieurs qui ne tenaient pas compte de la dépendance de la vitesse et de la profondeur dans l'hydraulique des chenaux de cours d'eau. Le terme des produits vectoriels ajouté au modèle modifie les pics prédits (densité) en réponse aux changements aléatoires de profondeur et de vélocité. Bien que le nouveau modèle améliore les prédictions, son efficacité dans la gestion des cours d'eau n'a pas encore été testée.

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RECENT developments in surface water consumptive use patterns, stream flow allocations, and various court rulings have made it imperative that aquatic ecologists quantify instream flow needs of biotic and abiotic components within the stream ecosystem. These components are linked to the ultimate point of interest, sport fishery production, by the energy transport function (macroinvertebrates as fish food) of each component in the system (Hynes 1970; Cummins 1979).

Fishery biologists or stream managers attempt to maintain stream systems in a "natural" state (that is, without perturbation and with productive populations). Threshold techniques (the $q$-10; the one in 10 years' flow) developed in the western United States (Waters 1976) do not consider life stage requirements of the fish component. However, Bovee et al. (1978) determined that lower trophic positions, generally less mobile, may have narrower tolerances to flow changes than the fish component. A streamflow management strategy, then, must include a flow to maintain invertebrates (often the primary fish food source) in order to assure optimum fish production.

Gore (1977, 1978) attempted to use a species or group of species as an indicator for instream flow evaluations based on the degree of responsiveness to flow changes. The "indicator" concept was based on the knowledge that certain organisms have a narrower tolerance than others for changes in discharge period and amplitude. These species responses were modeled using habitat suitability criteria represented by preference curves and surfaces for the variables of depth, current velocity, and substrate. This indicator concept was verified under natural conditions (Bovee et al. 1978). Major criticisms of this method and the complementary method for fish species (Bovee and Cochnauer 1977) arose from the inferred statistical reliability of the "probability-of-use" curves and the lack of a factor which includes the dependence of velocity and depth in channel hydraulics (Patten 1979). Our modeling efforts have been aimed at these criticisms. We hoped to produce a more sensitive model of responses to flow changes by the fish food component, the aquatic invertebrates.

## Methods

### HABITAT SUITABILITY

Weighted usable area (WUA) is the term which results from internal calculations of the PHABSIM model developed by the U.S. Fish and Wildlife Service, Cooperative Instream Flow Service Group (CIFSG) (Bovee and Milhous 1978; Milhous 1979). WUA is a function of suitability of velocity, depth, and substrate for a given organism:

$$(1) \qquad \text{WUA} = \sum_{i=1}^{n} A_i C_i$$

where: $A_i$ = surface area of a stream section element, $C_i$ = composite suitability of that stream element, $n$ = total number of elements being simulated. Each stream section element consists of all portions of a previously mapped stream section which have identical velocity, depth, and substrate characteristics. Composite suitability ($C_i$) is the product of suitabilities of each parameter in that stream element (velocity preference × depth preference × substrate preference). For the fish species, these suitabilities were derived from computed preference curves ("probability-of-use" curves; Bovee and Cochnauer 1977). This study was designed to fit macroinvertebrate data to similar equations.

TABLE 1. Arrangement of data from lowest to highest measured velocity. Same format was used for depth and substrate. $n$ = number of individuals. Species: *Nectopsyche lahontonensis*.

| Velocity (cm/s) | No. of samples | $n$ | $n$/0.1 m$^2$ |
|---|---|---|---|
| 0.0 | 2 | 0 | 0 |
| 3.05 | 0 | 0 | 0 |
| 6.1 | 2 | 0 | 0 |
| 9.15 | 4 | 1 | 0.25 |
| 12.2 | 1 | 0 | 0 |
| 15.25 | 2 | 0 | 0 |
| 18.3 | 0 | 0 | 0 |
| 21.35 | 3 | 2 | 0.67 |
| 24.4 | 0 | 0 | 0 |
| 27.45 | 3 | 2 | 0.67 |
| 30.5 | 6 | 21 | 3.5 |
| 33.55 | 5 | 7 | 1.4 |
| 26.6 | 3 | 7 | 2.33 |
| 39.65 | 2 | 23 | 11.5 |
| 42.7 | 8 | 23 | 2.9 |
| 45.75 | 7 | 9 | 1.29 |
| 48.8 | 2 | 1 | 0.5 |
| 51.85 | 8 | 13 | 1.63 |
| 54.9 | 1 | 1 | 1 |
| 57.95 | 5 | 12 | 2.4 |
| 61.0 | 8 | 28 | 3.5 |
| 64.05 | 3 | 6 | 2 |
| 67.1 | 5 | 8 | 1.6 |
| 70.15 | 6 | 14 | 2.33 |
| 73.2 | 3 | 8 | 2.67 |
| 76.25 | 4 | 52 | 13.0 |
| 79.3 | 7 | 18 | 2.57 |
| 82.35 | 3 | 31 | 10.33 |
| 85.4 | 5 | 24 | 4.8 |
| 88.45 | 8 | 94 | 11.75 |
| 91.5 | 12 | 137 | 11.42 |
| 94.55 | 6 | 32 | 5.33 |
| 97.6 | 2 | 9 | 4.5 |
| 100.65 | 4 | 11 | 2.75 |
| 103.7 | 3 | 8 | 2.67 |
| 106.75 | 0 | 0 | 0 |
| 109.8 | 1 | 1 | 1 |
| 112.85 | 1 | 7 | 7 |
| 115.9 | 4 | 42 | 8.4 |
| 118.95 | 3 | 11 | 3.67 |
| 122.0 | 4 | 5 | 1.25 |
| 125.05 | 0 | 0 | 0 |
| 128.1 | 2 | 4 | 2 |
| 131.15 | 2 | 3 | 1.5 |

Raw data from 465 quantitative benthic samples (Gore 1978 and unpublished data; Brusven 1977 and unpublished data) were analyzed for predominant species representing major functional groups (Merritt and Cummins 1978; Cummins and Klug 1979). Each data bit represented one sample and contained the following information: total number of individuals, number and kind of each species, depth (cm) at which the sample was taken, velocity (cm/s) measured at 0.6 ×

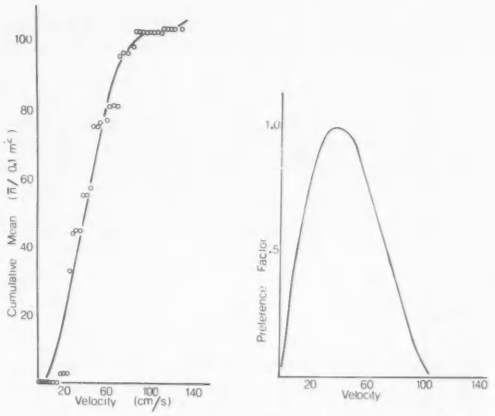



FIG. 1. Plot of cumulative mean number of individuals of *Nectopsyche lahontonensis* per increment of 5 cm/s of stream velocity (left) and its derived preference curve (right). $F = -6.7 + 51.1v^2 - 19v^3 + 2v^4$; $dF/dv + 102.1v - 57v^2 + 7.9v^3$.

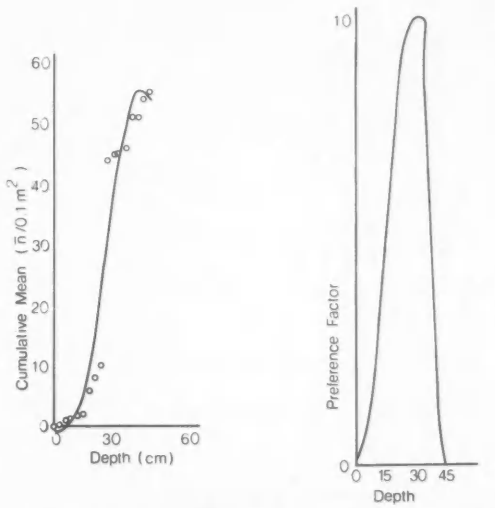



FIG. 2. Plot of cumulative mean number of individuals of *Nectopsyche lahontonensis* per increment of 3 cm of depth (left) and its derived preference curve (right). $F = -0.98 - 14.1d^2 + 99.6d^3 - 49d^4$; $dF/dd = -28.1d + 298.7d^2 - 195.9d^3$.

depth from the surface, and a single number characterizing the substrate (as in Gore 1978). The total number of samples at each increment of velocity, depth, and substrate were used in calculations, regardless of whether all samples contained the species being examined.

For brevity, the complete analysis for *Nectopsyche lahontonensis* (Banks) (Trichoptera), a collector–gatherer, will be shown.

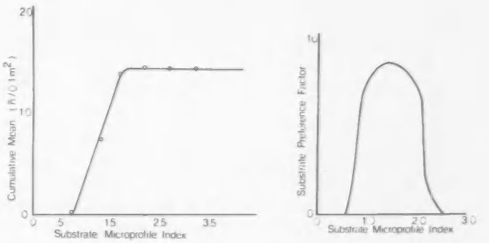



FIG. 3. Plot of cumulative mean number of individuals of *Nectopsyche lahontonensis* as a function of increasing substrate complexity ($m$) (as in Gore 1978) (left) and substrate preference curve (right) calculated by piecewise linear regression. $dF/dm = 1.61 + 4.11m - 1.85m^2 + 0.19m^3 - 0.9m^4$.

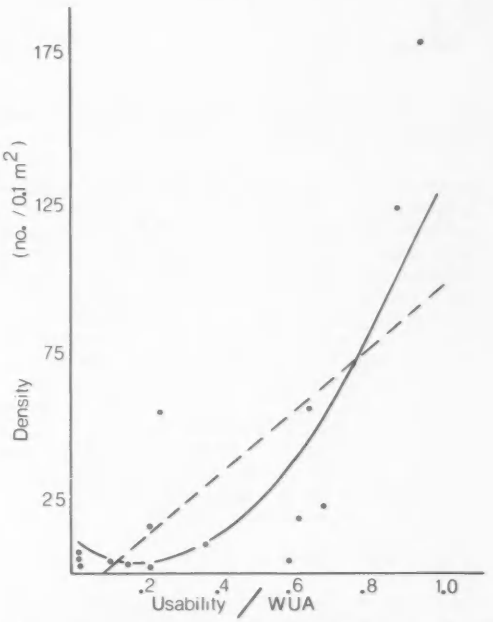



FIG. 4. Density of *N. lahontonensis* as a function of calculated habitat usability (WUA) ($P > 0.85$, for the curvilinear fit, solid line; linear fit, broken line).

The mean number of individuals per increment of habitat character was plotted as a cumulative function against each of the three variables of velocity, depth, and substrate (Table 1, Fig. 1, 2, and 3). This method was used to duplicate the analysis standard of the PHABSIM model. A fourth-order polynomial curve was fit to the data (Nie et al. 1975). The greatest incremental jump in cumulative means indicated the preference by the greatest number of individuals of *Nectopsyche*. The derivative of this cumulative mean curve provided a normalized curve with its peak at the preferred condi-

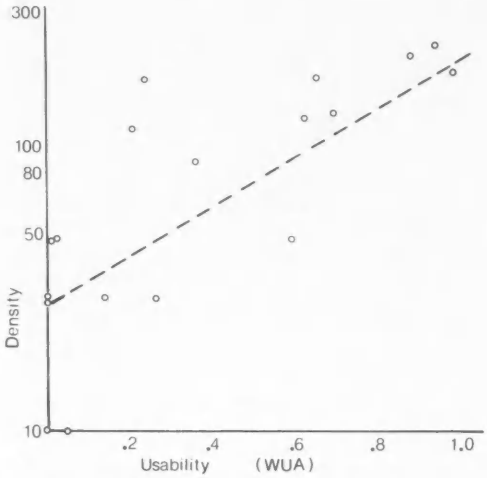



FIG. 5. $Log_{10}$ transformation of parabolic function (Fig. 4) for *N lahontonensis*. $r^2 = 0.71$; $P > 0.85$.

tion of current velocity, depth, or substrate (Fig. 1, 2, and 3). In some cases, (as in Fig. 3), when the ascending limbs of the cumulative mean curves were derived from less than 5 data points, a piecewise linear regression was used to calculate the preference curve (Mendenhall 1975). The values of actual density were plotted as a function of habitat usability (WUA) and analyzed using a simple linear regression model (Seber 1977). If the $r^2$ value was less than 0.60, a curvilinear plot was used to improve the fit of the actual data to the predictive function (Fig. 4). This curvilinear fit, often parabolic in form, was log-transformed to give a linear interpretation of the function as a predictor of invertebrate densities (Fig. 5). The log transformation helped to eliminate predictive problems (see Fig. 4 in which the curvilinear plot indicates that no usable habitat has higher densities than 20% usable habitat) encountered with the parabolic function.

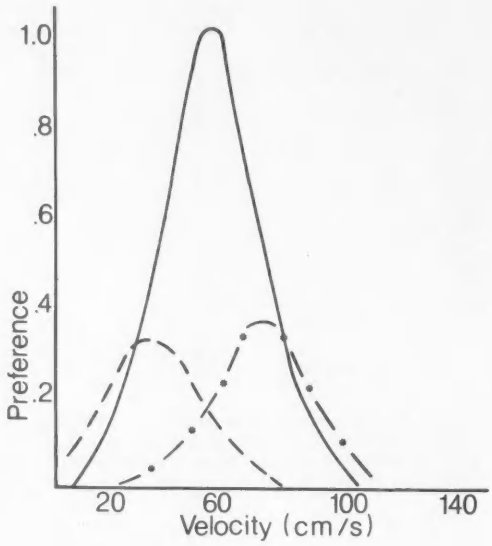
## VELOCITY AND DEPTH DEPENDENCIES

We chose the method of exponential polynomial curve fitting to test the independence of velocity and depth as the model related to invertebrate densities. This model was used as it is a statistically understood, non-negative distribution that has been shown to fit empirical data (Matz 1978). If the cross-product correlation coefficient ($a_5$):

$$(2) \qquad \exp\{-(a_1v + a_2d + a_3v^2 + a_4d^2 + a_5vd)\}$$

was not significantly different from 0, independence of velocity ($v$) and depth ($d$) would be shown.

We analyzed each species by constructing histograms of the frequency of occurrence of individuals for velocity and depth. A multiple regression (Nie et al. 1975) was used to estimate the initial coefficients for each term ($a_n$) in the exponential polynomial (equation 2). A quadratic function was used when the relative frequency of the histogram showed a unimodal distribution.



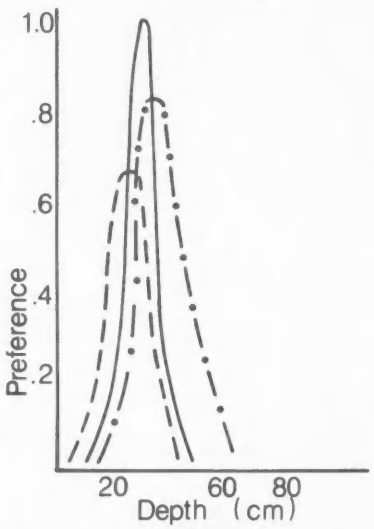




FIG. 6. Exponential polynomial velocity and depth curves for *N. lahontonensis*. $f'' = \frac{1}{414.7} \exp -(-0.448v - 12.8d + 1.77v^2 + 12.6d^2 - 5.92vd)$. For solid line (*upper*), $d = 27.4$ cm; broken line (*upper*) $d = 15.2$ cm; broken and starred line (*upper*), $d = 38.1$ cm; solid line (*lower*) $v = 48.8$ cm/s; broken line (*lower*), $v = 30.5$ cm/s; broken and starred line (*lower*), $v = 61.0$ cm/s.

The exponential curve model described the joint distribu-

tion of observations for velocity and depth. Results were expressed as a response surface for the species where:

$$(3) \quad f' = \exp\{-p(v,d)\}$$

which yielded a probability distribution.

The partial differentials of $f'$ were calculated to determine the value of $v$ and $d$ that represented preferred habitat conditions:

$$(4) \quad \frac{\partial f'}{\partial v} = a_1 + 2(a_3 v) + a_5 d = 0$$

and

$$(5) \quad \frac{\partial f'}{\partial d} = a_2 + 2(a_4 d) + a_5 v = 0$$

The simultaneous solution of the partial differentials for $v$ and $d$ were substituted back into the original equation (2) to give the normalization value, $N$.

With the normalization value, the equation:

$$(6) \quad f'' = \frac{1}{N} \exp\{-(a_1 v + a_2 d + a_3 v^2 + a_4 d^2 + a_5 v d)\}$$

calculated a preference response curve, ranging from 0 to 1.

Plots of the function, $f''$, while holding either depth or velocity constant, were calculated (Fig. 6). Analysis of the deviations of the cross-product terms in these response curves allowed us to determine the significance of the interactions of velocity and depth.

## Results and Discussion

### Habitat Suitability

Habitat preferenda for 19 aquatic invertebrates are presented in Table 2.

TABLE 2. Velocity, depth, and substrate preferenda for selected aquatic invertebrates. Determined by PHABSIM methods and an 85–100% preference range (*) determined by cross-product polynomial curves.

| Species | Functional group | Velocity (cm/s) | Depth (cm) | Substrate (Gore 1978) |
|---|---|---|---|---|
| *Alloperla* sp. | Collector–gatherer | 12.2–85.4 | 5.2–49.7 | 3–5 |
| | | 42.7–61* | 20.4–35.7* | 3.75–4.15* |
| *Rhithrogena hageni* | Collector–scraper | 29.6–126 | 9.2–48.8 | 0.85–3.5 |
| | | 76.3–106.8* | 28.7–38.4* | 1.6–2.6* |
| *Symphitopsyche bifida* | Collector–netspinner | 13.1–118.9 | 4.9–48.8 | 0.55–3.35 |
| | | 64.1–91.5* | 28.7–39.7* | 1.86–2.6* |
| *S. recurvata* | Collector–netspinner | 76.3–94.6* | 33.6–42.7* | 2.75–3.5* |
| *S. occidentalis* | Collector–netspinner | 12.8–122 | 0–45.8 | 0.75–3.5 |
| | | 39.7–58* | 24.4–33.6* | 1.5–2.2* |
| *Baetis hageni* (= *parvus*) | Collector–scraper | 10.7–131.2 | 3.1–42.7 | 0.55–3.3 |
| | | 30.5–65.6* | 14.3–23.2* | 1.85–2.5* |
| *Cheumatopsyche* spp. | Collector–netspinner | 10.7–115.9 | 3.1–48.8 | 0.80–3.3 |
| | | 39.7–77.8* | 23.8–38.4* | 1.86–2.6* |
| *Baetis insignificans* | Collector–scraper | 6.4–129.6 | 0–44.2 | 0.75–3.5 |
| | | 25.9–65* | 9.5–24.1* | 1.5–2* |
| *Stenonema terminatum* | Scraper | 9.2–90.1 | 2.4–41.2 | 0.85–2.8 |
| | | 25.9–51.9* | 19.2–28.7* | 1.3–1.9* |
| *Ephemerella inermis* | Collector–gatherer | 13.1–131.2 | 3.1–43.5 | 0.37–3.5 |
| | | 51.9–91.5* | 14.3–28.7* | 1.5–2.23* |
| *Ceraclea* sp. | Collector–shredder | 13.1–113.8 | 4.9–47.3 | 0.84–3.35 |
| | | 39–65* | 14.3–24.1* | 1.48–2.23* |
| *Tricorythodes minutus* | Collector–gatherer | 0–94.6 | 8.5–47.9 | 0.95–3.1 |
| | | 25.9–51.9* | 14.3–24.1* | 1.49–2.23* |
| *Choroterpes albiannulata* | Scraper | 0–115.9 | 4.3–47.9 | 1.12–2.75 |
| | | 51.9–90* | 19.2–28.7* | 1.5–2.1* |
| *Stenelmis crenata* (adult) | Scraper–collector | 13.1–109.8 | 9.8–40.6 | 0.95–2.9 |
| | | 32–58* | 19.32–28.7* | 1.12–2.23* |
| *Stenelmis crenata* (larvae) | Scraper–collector | 13.1–103.7 | 9.5–42.4 | 0.40–3.0 |
| | | 25.9–65* | 14.3–28.7* | 1.5–2.5* |
| *Chloroperla* sp. | Predator | 13.7–114.4 | 5.5–61 | 0.80–3.4 |
| | | 51.9–77.8* | 14.3–19.2* | 1.7–2.23* |
| *Acroneuria abnormis* | Predator | 13.1–125.1 | 0–48.8 | 0.85–3.54 |
| | | 51.9–91* | 14.3–24.1* | 1.5–2.23* |
| *Dugesia tigrina* | Collector–omnivore | 13.1–85.4 | 6.7–47.9 | 0.95–3.0 |
| | | 25.9–51.9* | 14.3–28.7* | 1.5–2.23* |

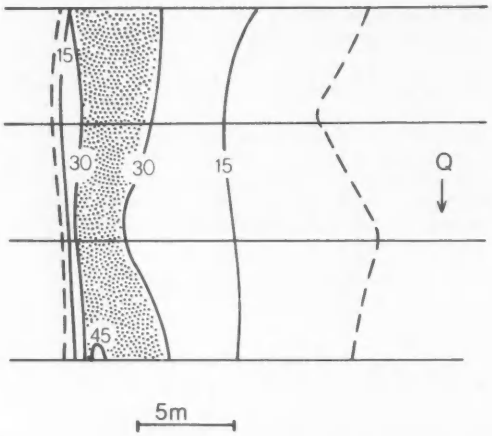



FIG. 7. Isodepth (cm) map of a section of the Tongue River in Montana, USA. Derived from measurements along four transects. Adapted from Bovee et al. (1978). Broken lines indicate banks. Stippled areas represent the region of 100% suitability for *N. lahontonensis*. Discharge ($Q$) = 1.58 $m^3/s$.

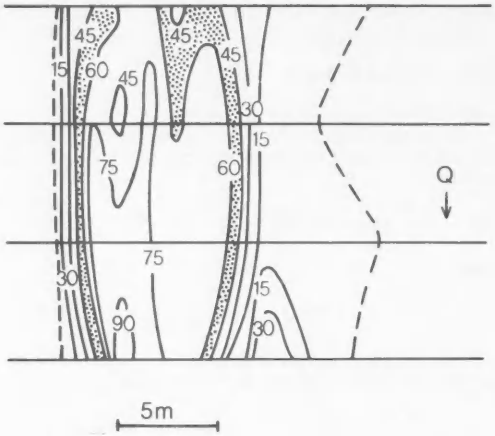



FIG. 8. Isovelocity (cm/s) map of a section of the Tongue River in Montana, USA. Derived from measurements along four transects. Adapted from Bovee et al. (1978). Broken lines indicate banks. Stippled areas represent 100% velocity suitability for *N. lahontonensis*. Discharge ($Q$) = 1.58 $m^3/s$.

Examination of density versus habitat usability showed that the logarithmic plot appeared to be a good predictor of density for *Nectopsyche* (Fig. 4 and 5). The log-transformed plots for other species showed equal reliability ($r^2 \geq 0.65$, $p \geq 0.90$).

Several linear models to show fish standing crop as a function of specific water quality parameters (including flow) have been formulated (Ryder 1965; Binns and Eiserman 1979). The nonlinear relationships we derived for *Nectopsyche* (Fig. 4 and 5) and for other aquatic invertebrates imply that as habitat quality decreases, invertebrate standing crop (density) decreases disproportionately. A management strategy which accounts for both benthos and fish will likely be more effective than one accounting for either component alone.

Bovee et al. (1978) and Stalnaker (1979) have shown that certain flow requirements override all others during a given time period. For example, adequate flow requirements to maintain fish and benthos are not sufficient to provide flushing flows during spring runoff. Similarly, flows adequate to maintain benthic fauna would not be sufficient to maintain shallow, high-water velocity gravel beds for salmonid spawning. An annual flow allotment will not always consider these temporal changes in flow requirements. Allotments based on shorter time increments (monthly or at least quarterly) are advisable. In this manner, WUA curves can be used to determine "special need" flows for a time increment. The annual allotment, as a sum of the monthly or quarterly allotments, will be most effective.

Certainly there are many more variables than velocity, depth, and substrate influencing distribution of benthic invertebrates (Minshall and Minshall 1978; Vannote et al. 1980). The general model we have presented does explain a significant amount of the variability in densities. Considering the constraints that any model has on describing reality, the model is useful in estimating flows necessary to maintain

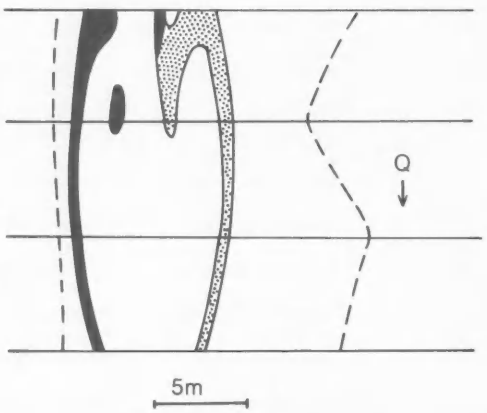



FIG. 9. Composite isodepth and isovelocity map derived from Fig. 4 and 5. Broken lines indicate banks. Solid black areas are regions of 100% composite suitability (WUA). Stippled areas represent 85% suitability. $Q$ = 1.58 $m^3/s$. Total area of 100% suitable habitat = 9.3 $m^2$. WUA = 0.16.

adequate habitat conditions. Many independent research teams have determined the WUA method for fisheries to be adequate for management of flows for fish populations (Wesche and Rechard 1980). We suggest that incorporation of benthos flow predictions will serve to increase the sensitivity of the management program. This model does assume that changes in chemical factors will be minimal with a new flow strategy. The use of an integrated system of monitors (e.g. the biotic condition index: Winget and Mangum 1979) during

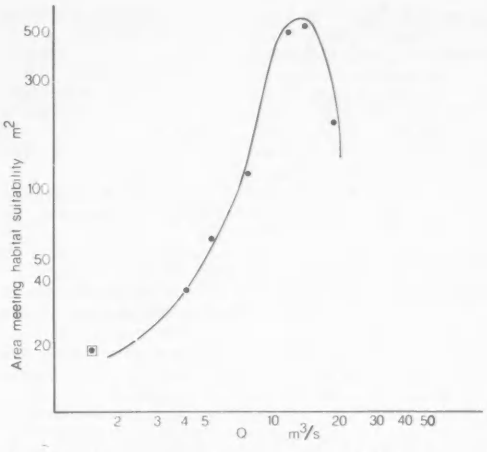



FIG. 10. Plot to predict optimum discharge for *N. lahontonensis* at the area mapped for Fig. 7, 8, and 9. Point within square is derived from composite map, Fig. 9.

periods of altered flow could be used to assess the degree of these changes.

As an example of the application of the model, depth and velocity contour maps of a typical stream section can be produced for a number of low and high discharge conditions (as in Fig. 7 and 8). A composite map (Fig. 9) will reveal the WUA value of that section at each discharge. A plot of WUA values and discharges for that section (Fig. 10) will predict a peak or optimum discharge condition. If this plot is for an indicator species (Gore 1977, 1978; Bovee et al. 1978), a stream manager has a clear set of flow rates and predicted i.npacts which can be used to determine potential losses from an altered flow regime.

When analyzing other aquatic invertebrates (Table 2), we noticed some overlap in habitat preferenda within functional groups and between similar species. This is a manifestation of the model's ignorance of temporal and spatial separation of many related forms (Gore 1978). Management of flows must account for temporal changes in community composition and the existence of several distinct communities (and, possibly, indicator species) within a watershed system (Vannote et al. 1980). Effective use of this method implies initial collecting and flow measurement regimes which cover the length of the managed portion of the river for a period of a year preceeding estimation of flow alternatives.

### VELOCITY AND DEPTH DEPENDENCIES

The results of tests of the deviation of the cross products in the exponential polynomials (Sokal and Rohlf 1969), for five test species, are shown in Table 3. All tests were statistically significant. Thus, the cross-product term does affect the prediction of densities at various velocities and depths. Visual inspection of the curves (Fig. 6) showed shifts in the peak of preference as velocity and depth, in combination, changed.

## Conclusions

Instream flow needs based on physical habitat conditions can be accurately predicted by our method of determining the effect of flow manipulations on fisheries and other aquatic organisms. The method of predicting a regulated flow regime, based on amount of WUA per discharge, has shown to be effective in producing adequate flow recommendations (Stalnaker 1979). The major criticisms of the model do not appear to be critical constraints. This study indicated that habitat preferences are measurable and reasonably predictable.

The exponential polynomial yielded response surfaces that seemed to represent better the organism's selection of variables than the incremental curve-fitting technique of PHABSIM (Bovee and Cochnauer 1977). However, the maximum values of each curve generated in the family of velocity and depth curves (Fig. 6) fell within 10% of the values calculated by the WUA — contour map methods. The shift in peak response (cross-product analysis) will be important only if the resulting WUA show large disparities related to densities encountered in stream ecosystems. At present, analysis of potential disparities is not possible because of lack of field data. Results of WUA calculations, performed by using PHABSIM, have been shown to be significantly correlated with field observations of fish biomass in tests completed to date (K. D. Bovee and C. B. Stalnaker, CIFG, personal communication). With the data on hand, the incremental curve-fitting methods (Fig. 1 through 5) are, likewise, adequate to predict approximate densities of aquatic macroinvertebrates. Thus, our proposed exponential polynomial

TABLE 3. Cross-product values from *t*-statistic testing exponential polynomials (as in Fig. 6) (Sokal and Rohlf 1969).

| Species | df | $u^a$ | SE[b] | $t$ | $\alpha$ |
|---|---|---|---|---|---|
| *Symphitopsyche recurvata* | 161 | 1.44 | 0.297 | 4.84 | <0.01 |
| *Baetis hageni* | 161 | 2.62 | 0.433 | 6.06 | <0.01 |
| *Nectopsyche lahontonensis* | 161 | 5.92 | 0.896 | 6.61 | <0.01 |
| *Acroneuria abnormis* | 161 | 0.72 | 0.260 | 2.78 | <0.01 |
| *Baetis insignificans* | 161 | 2.17 | 0.371 | 5.85 | <0.01 |

[a] $u$ = observed deviation of $a_5$.

[b] SE = standard error of the solution term on the cross product ($v \times d$) (equation 6).

method may be a "fine tuning" function to allow a narrower confidence limit on regulated flow recommendations. This could be tested by dividing the dependence factor calculated with the exponential polynomial by the dependence factor ($a_S$) of velocity and depth from real data (in an experimental reach). If the quotient equalled 1.0, all dependence would be attributable to the actual distribution of velocity and depth in the channel.

Indicator invertebrate species, on a site-specific basis, can be determined by visual observation of preference curves. Those species with the narrowest range of preferenda, yet contained within the preference ranges of the greatest number of other community members, can be used to derive WUA-discharge predictions. In combination with flow requirements for spawning and maintenance of important game fish, flow requirements for flushing of accumulated detrital material, and the flow to maintain good riparian growth, the addition of predictions of flows for aquatic macroinvertebrates should result in effective streamflow management.

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# Initial Colonization of Artificial Substrate: Community Development and Structure Studied by Scanning Electron Microscopy

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HUDON, C., AND E. BOURGET. 1981. Initial colonization of artificial substrate: community development and structure studied by scanning electron microscopy. Can. J. Fish. Aquat. Sci. 38: 1371 – 1384.

The initial phases of subtidal and intertidal community development were observed using scanning electron microscopy on samples from plastic panels immersed monthly in the St. Lawrence Estuary.

Bacteria and diatom populations were quantitatively evaluated on samples collected from May to November, 1978. The pattern of immersion and/or periodical emersion according to the level greatly influenced the community structure. Subtidal panels (−5.0 m) were rapidly colonized by bacteria while diatoms settled 2−7 wk later, depending on the season. *Cocconeis* spp. and *Amphora* spp. were the major diatom colonizers until mid-August. In September, *Synedra tabulata* settled on the panels. Until mid-September, all dominant species formed well-defined, generally monospecific clumps. In contrast with *Cocconeis* spp. and *Amphora* spp., which lie horizontally on the surface, *S. tabulata*, which is needlelike in shape, formed erect fan-shaped colonies. Other late invaders possessed a mucus stalk, raising themselves from the surface and thus better utilizing the vertical dimension. Clump overlap and increased species interactions occurred with higher cell densities. In the intertidal zone bacteria settled after 8−12 wk while *Achnanthes brevipes* var. *parvula* appeared after 20 wk, the only diatom species able to resist semidiurnal emersion. The ability of the panels to retain water through detritus and irregularities is probably the main factor allowing the development of this community. Panels emersed only at spring tides (monthly) were rapidly colonized by bacteria, and heavy diatom settlement occurred within 4 wk. Successive monthly emersions eliminated or strongly reduced diatom populations, which were replaced by filamentous (Ectocarpaceae) algae. The three types of communities are compared and the strategic advantage of upright forms is discussed in relation to population density, light availability, and detrital cover.

*Key words*: St. Lawrence Estuary, artificial substrate, community structure, community development, *Cocconeis* spp., *Synedra tabulata*, *Amphora* spp., diatoms

HUDON, C., AND E. BOURGET. 1981. Initial colonization of artificial substrate: community development and structure studied by scanning electron microscopy. Can. J. Fish. Aquat. Sci. 38: 1371 – 1384.

Le développement initial des communautés infralittorales et intertidales a été observé en microscopie électronique à balayage sur des plaques de plastique immergées dans l'estuaire du St-Laurent. Les populations de bactéries et de diatomées ont été évaluées quantitativement entre mai et novembre 1978. Les patrons de colonisation varient selon que l'émersion est semi-diurne, mensuelle ou absente. Dans l'étage infralittoral, les bactéries colonisent rapidement les plaques, tandis que les diatomées se fixent de 2−7 semaines plus tard, selon la saison. *Cocconeis* spp. et *Amphora* spp. dominent la flore jusqu'à la mi-août. En septembre, *Synedra tabulata* colonise les plaques. Durant cette période, les espèces dominantes forment des agrégats monospécifiques distincts, généralement isolés. Contrairement aux espèces pionnières, qui reposent horizontalement sur le substrat, *S. tabulata* forme des colonies dressées en forme d'éventails, alors que d'autres espèces utilisent un pédicelle muqueux pour se soulever au dessus du substrat. La superposition des espèces survient avec l'augmentation de densité. Dans l'étage intertidal, les bactéries se fixent 8−12 semaines après l'immersion, tandis qu'*Achnanthes brevipes* var. *parvula*, la seule diatomée résistante à l'émersion semi-

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diurne, n'apparaît qu'après 20 semaines. La présence de détritus sur le substrat est l'un des principaux facteurs de rétention d'eau, permettant indirectement la croissance des diatomées. Les plaques émergées aux marées de vives-eaux seulement (mensuellement) sont rapidement colonisées par les bactéries et les diatomées. Par contre, les émersions successives réduisent, voire éliminent les diatomées, qui sont remplacées par les ectocarpales. Les trois types de communautés sont comparés et l'avantage stratégique du développement vertical est examiné en relation avec la densité des populations, la disponibilité de lumière et la présence des détritus.

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In most studies of early phases of colonization of solid substrates, bacteria and diatoms are considered separately mainly because of the differing requirements of methodological approach. Bacteria are usually stained and examined in light or epifluorescence microscopy whereas diatoms are usually scraped off their substrate and later examined using light microscopy. Although of considerable interest, such studies allow consideration of only the physicochemical tolerances and the seasonal variations of species abundance. Because they ignore spatial relationships, they result in a very incomplete view of the organization of community.

An overall view of the taxa present without altering their original position and distribution can be obtained using light microscopy of intact populations settled on glass slides (Bélanger and Cardinal 1977). However, this technique is restricted by the substrate nature and multilayered cell cover. These limitations were overcome by using scanning electron microscopy (SEM) to study the early phases of settlement on a number of natural and artificial substrates. The flora associated with the surface of various aquatic macrophytes, such as *Ascophyllum nodosum* (Cundell et al. 1977), *Chara* sp. and *Potamogeton natans* (Allanson 1973), *Spartina alterniflora* (Gessner et al. 1972), and *Zostera marina* (Sieburth and Thomas 1973), was studied. Surface colonization of marine plants has been reviewed by Sieburth et al. (1974). Nonliving organic substrates such as driftwood were also investigated using SEM (Brooks et al. 1972; Cundell and Mitchell 1977) and a comparison between natural and artificial substrates was made by Paul et al. (1977), using sycamore leaf and polyurethane foam. Investigations of various inert natural surfaces such as rock (Perkins and Kaplan 1978) and sand grains (Weise and Rheinheimer 1978) indicated preferential settlement and development in cracks and holes of the substrate surface. Di Salvo and Daniels (1975) used glass slides to examine bacterial attachment.

Although these studies gave important information about the community structure and organization, they provided no quantitative analysis of the sequence of colonization by bacteria and diatoms. As pointed out by McIntire and Moore (1977), few studies give a comprehensive view of the biological interactions occurring among diatom taxa as well as between the diatoms and other groups of plants and animals. Accordingly, this study examines quantitatively the community development and biological interactions occurring in a slow-growing, low-density periphytic assemblage.

## Study Area

The study area, a 90-km section of the south shore of the Lower St. Lawrence Estuary (Fig. 1), is influenced by strong semidiurnal tides ranging from 1.1 to 4.6 m (Canadian Hydrographical Service 1978).

Salinity and temperature showed no strong spatial variation or gradients throughout the area. At any given time, salinity and temperature never varied more than 2‰ and 3°C between any two stations of similar depth. Water transparency ranged from 2 to 5 m (Nota and Loring 1964), depending on mixing.

Seasonal variations of salinity and temperature occurring during the sampling period were much more pronounced. From early June to early November, salinity steadily increased from 24 to 29‰. Surface water temperature increased from 9°C in early June to a maximum of 15°C in early August, and thereafter decreased gradually to 2°C in late October. At 5-m depth, water temperature varied from 3 to 9°C in August, then decreased to 2°C in late October.

## Material and Methods

Sampling stations were established at Leggatt's Point, Pointe Mitis, and Les Ilets in the intertidal and subtidal zones (Fig. 1). The first two stations were located on the exposed and protected sides, respectively, of the same headland whereas at Les Ilets the sheltered side of a pier was used.

Black, flexible, and nontoxic Conoflex® (Pionner Plastic, division of Lof Plastic Inc.) plastic sheets were used as substrate throughout the study. This material is resistant to the various solvents used as well as to low temperatures. Its surface contour shows fine ridges about 15 μm wide and 3 μm deep which are very suitable for initial colonizers.

Fifty-seven 400-cm$^2$ Conoflex® panels were placed vertically on the various collectors. Intertidal collectors were made of large plywood sheets attached either to a vertical support anchored in the tidal zone as in Bourget and Lacroix (1971) or directly onto the side of a pier. Subtidal collectors were made out of welded iron bars which held the panels 0.5 m above the bottom. The subtidal collectors were sampled by SCUBA diving.

One intertidal and one subtidal collector were placed at Leggatt's Point and Pointe Mitis stations. Les Ilets pier held a vertical row of panels from the low-water level (chart datum) and above. The vertical position of each collector is given in Table 1.

Each collector contained a series of panels which remained in position for periods ranging from 3 to 22 wk. At each site, four panels were present during the entire sampling period. Three other panels were immersed at monthly intervals until the last collection in November, and extra panels were also added irregularly. Subtidal stations were sampled at fort-

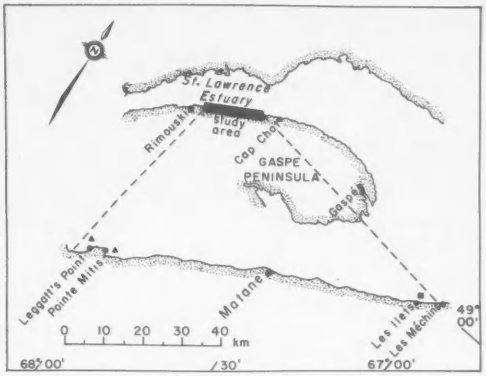


FIG. 1. Location of the stations in the lower St. Lawrence Estuary.

nightly intervals and all others were sampled at weekly intervals. Table 1 gives details of total number of panels and samples at each station.

All samples were obtained by punching out 2 × 2 cm fragments from the panels and each sample thus represented 1% of the panel area. Sample size was limited by the size of the microscope chamber. Because during the field work a strong influence of tidal level was observed on distribution, all the panels were sampled in the top, middle, and lower portions. The exact location of each sample at random within a given portion was noted using a coordinate grid. SEM preliminary observations of intertidal panels showed that marked variations of the populations occurred within a vertical range as small as 15 cm. Thus, all samples used for temporal comparisons in the intertidal came from the same level. Such spatial variation was not observed in the subtidal zone, so there, all three replicate samples were used to describe populations on a given panel. Procedure was as follows. Immediately after collection, all samples were immersed in filtered seawater and taken to the laboratory. They were then fixed for the SEM using phosphate-buffered (pH 7.2) glutaraldehyde 2.5% for 2 h and dehydrated in a graded series of ethanol solutions (25%, 50%, 75%). Samples were kept in 75% ethanol until final SEM preparation. Whole panels and additional duplicate samples were directly fixed in 75% ethanol except when colonized by invertebrate larvae. The latter were prepared for the SEM as indicated above. Before SEM observation, samples were transfered to 99% ethanol, freeze-dried for 12 h, mounted with nail polish on aluminium stubs, and coated with gold–palladium. Observations were carried out with a SEM ETEC-AUTOSCAN at 30 kV and 0° angle beam. The fixation and drying procedures were selected for their reliability for the preservation of surface-associated microorganisms (Garland et al. 1979). Cell enumeration in the residual rinsing solutions showed an insignificant number of cells lost due to manipulations.

Dry weights of total organisms colonizing individual panels were obtained by scraping freeze-dried samples. The scraped material was then weighed on a Cahn balance to the nearest 0.01 mg.

Samples were analyzed as follows. All diatom taxa were individually characterized during the counting procedure. Taxa observed in less than 5% of the samples were not considered further in the analysis. The diatoms were identified with characteristics seen with the SEM and light microscope using monographs of Boyer (1927a, b), Husted (1930), Cleve-Euler (1951–55), Hendey (1964), and Patrick and Reimer (1966). All identifications were later verified against collections at the Philadelphia Academy of Sciences and checked by Dr Ruth Patrick.

A statistically valid estimate of bacteria and diatom populations present on a sample was obtained by counting individuals at 2000× (1000 $\mu m^2$) for bacteria and at 500× (38 000 $\mu m^2$) for diatoms. The distribution of organisms on each sample was determined by fitting a theoretical model (positive binomial, Poisson series, or negative binomial) to the counts of randomly selected microscopic fields at the appropriate magnification. For each sample, bacteria were counted on 5–10 quadrats, depending on density. For diatoms, up to 250 microscopic replicate observations were necessary to give an accurate count (precision on the mean >80%).

## Results

### Species Composition and Spatial Distribution

We considered bacteria to include all rods and cocci forms, and we made no attempt at strain identification. The cells were easily visible on the substrate and no slime formation was observed. The distribution of bacteria was tested with a Kolmogorov–Smirnov test applied to the quadrat counts. The results given in Table 2 indicate a random distribution of bacteria on intertidal and subtidal panels at both high and low densities.

We identified 20 taxa of diatoms belonging to 17 genera (Table 3). Major species abundance and composition were quite similar at the two subtidal stations (Leggatt's Point and Pointe Mitis). It can also be observed that the lower intertidal station of Les Ilets had more species in common with the subtidal stations than the other intertidal stations. The total number of species diminished with increasing tidal height.

TABLE 1. Description of the stations and sample characteristics.

| Station | Leggatt's Point | | Pointe Mitis | | Les Ilets |
|---|---|---|---|---|---|
| Location of collector | Intertidal | Subtidal | Intertidal | Subtidal | Pier side |
| Vertical range (m) | +2.7 to 2.3 | −5.0 | +1.6 to 1.1 | −5.0 | +2.7 to 0.3 |
| Total no. of panels immersed | 9 | 13 | 13 | 12 | 10 |
| Total no. of samples | 145 | 143 | 144 | 82 | 231 |
| No. of sampling periods | 14 | 6 | 14 | 6 | 12 |

TABLE 2. Examples of random distribution of bacteria from intertidal and subtidal samples of different densities, by mean of Kolmogorov–Smirnov test. The $N$ replicate counts were obtained from 2000× (1000 $\mu m^2$) enlargements using the SEM. $^{**}P < 0.001$.

| | Densities | $N$ | Minimum | Maximum | Mean | Standard error | Maximum deviation |
|---|---|---|---|---|---|---|---|
| Intertidal | High | 25 | 208 | 846 | 554.2 | 65.7 | 0.105** |
| | Low | 25 | 80 | 599 | 328.56 | 44.9 | 0.073** |
| Subtidal | High | 29 | 338 | 858 | 559.0 | 48.8 | 0.092** |
| | Low | 30 | 67 | 187 | 111.7 | 10.9 | 0.058** |

TABLE 3. Presence (%) of the different taxa of diatoms found at Leggatt's Point, Pointe Mitis, Les Ilets, and the intertidal collectors of the same stations. Percentages of presence were calculated from numbers of samples at each station where diatoms were present.[a]

| | Subtidal (−5.0 m) | | Intertidal Low (+0.3 to +0.7 m) | Intertidal High (>+1.0 m) |
|---|---|---|---|---|
| Taxa | Leggatt's Point % | Pointe Mitis % | Les Ilets % | Leggatt's Point Pointe Mitis Les Ilets % |
| *Cocconeis costata* | 95 | 90 | 75 | 25 |
| *Synedra tabulata* | 70 | 71 | 25 | 0 |
| *Achnanthes pseudogroenlandica* | 70 | 71 | 75 | 0 |
| *Amphora pusio* | 63 | 96 | 25 | 5 |
| *Synedra investiens* | 60 | 86 | 88 | 15 |
| *Gomphonema kamtschaticum* | 60 | 29 | 63 | 5 |
| *Biddulphia aurita* | 49 | 19 | 0 | 0 |
| *Navicula* spp. | 47 | 71 | 75 | 0 |
| *Cocconeis scutellum* | 40 | 14 | 13 | 5 |
| *Amphora costata* | 37 | 0 | 13 | 0 |
| *Diploneis* spp. | 37 | 5 | 0 | 0 |
| *Coscinodiscus* spp. | 33 | 5 | 13 | 0 |
| *Rhoicosphenia curvata* | 23 | 19 | 13 | 5 |
| *Navicula crucicula* | 23 | 19 | 0 | 0 |
| *Gyrosigma arcticum* | 5 | 24 | 13 | 0 |
| *Achnanthes brevipes* var. *parvula* | 0 | 0 | 75 | 80 |

[a]Species present on less than 10% of the samples bearing diatoms, at the two subtidal stations: *Rhabdonema arcuatum*, *Fragilaria construens*, *Nitzschia paradoxa*, *Gyrosigma fasciola*, *Thalassiosira nordenskioldii*, *Chaetoceros atlanticus*, and *Skeletonema costatum*. The last three were damaged planktonic species.

TABLE 4. Frequency (%) of dominance of the major diatom species found at Leggatt's Point, Pointe Mitis, and Les Ilets. The percentage of dominance was computed from the number of samples where the species was present. A species was considered dominant when it reached at least 40% of the number of diatom cells present.

| | Subtidal | | Low intertidal | High intertidal |
|---|---|---|---|---|
| Taxa | Leggatt's Point | Pointe Mitis | Les Ilets | Leggatt's Point Pointe Mitis Les Ilets |
| *Cocconeis costata* | 57 | 32 | 50 | — |
| *Synedra tabulata* | 87 | 50 | — | — |
| *Amphora pusio* | — | 24 | — | — |
| *Synedra investiens* | — | — | 14 | — |
| *Achnanthes brevipes* var. *parvula* | — | — | — | 88 |
| Total no. of samples analyzed | 50 | 25 | 22 | 51 |
| Total no. of species | 23 | 18 | 14 | 7 |
| No. of samples with diatoms | 43 | 21 | 8 | 20 |
| No. of dominant species | 2 | 3 | 2 | 1 |

TABLE 5. Results of the goodness of fit test applied to the Poisson series and negative binominal distribution for two densities of the dominant diatoms species. $^{**}P < 0.01$; $^{***}P < 0.005$.

| | $N$ | $\bar{x}$ | $K$ | Poisson series | Negative binomial |
|---|---|---|---|---|---|
| *Synedra tabulata* | 50 | 36.28 | 1.33 | $0.31\times10^5$ | 15.53** |
| | 60 | 5.55 | 0.44 | $1.65\times10^5$ | 18.18*** |
| *Cocconeis costata* | 70 | 10.84 | 0.42 | $12.5\times10^5$ | 4.3** |
| | 60 | 5.27 | 0.79 | $0.44\times10^5$ | 11.77** |
| *Amphora pusio*[a] | — | — | — | — | — |
| | 60 | 2.37 | 0.22 | $14\times10^9$ | 1.47 |
| *Achnanthes brevipes* var. *parvula* | 60 | 15.98 | 0.78 | $39\times10^5$ | 4.49** |
| | 60 | 5.03 | 0.70 | $9.58\times10^5$ | 4.41** |

[a]Only low densities were observed.

Only *Achnanthes brevipes* var. *parvula* was limited to intertidal stations, where it was very common.

Five dominant species were observed (Table 4). *Cocconeis costata* was present at all stations (Table 3) and was dominant at lower sampling sites (−5, 0, and +0.5 m). *Synedra tabulata* and *Amphora pusio*, two other common species (Table 3), were dominant at −5.0 m only. *Synedra investiens*, a species common at all stations, reached dominance in the low intertidal zone (+0.5 m at Les Ilets) while *A. b.* var. *parvula* dominated higher level sites.

Diatom distribution was first examined using counts from random microscopic field areas at 500×. Highest and lowest densities of four dominant species of diatoms were fitted to the Poisson and negative binomial theoretical distributions. The results given in Table 5 indicate that the negative binomial model fits the data best, thus indicating a strong and constant contagious distribution of cells on the substrate. The precision on the estimated population mean was maintained at a tolerable error of 20%, using the following equation to determine the appropriate number of subsamples:

$$N = \frac{1}{D^2}\left[\frac{1}{\bar{x}} + \frac{1}{K}\right]$$

where $N$ is the number of replicate observations necessary to attain a fixed percentage of confidence ($D$ = 80%) on the sample's mean. A preliminary sampling provides estimate values of the mean ($\bar{x}$) and of the second parameter of the negative binomial distribution ($K$) (Elliott 1971).

Characteristic aggregated distributions of dominant species of diatoms are shown in Fig. 2. At first, all clumps were strongly monospecific, separated by large bare areas. *Cocconeis costata* (Fig. 2A) and *Amphora* spp. were usually lying with the raphe facing the substrate and had a tendency to occupy the ridges in the substrate. The cells of *S. investiens* and *S. tabulata* were both similar in shape but they showed very different patterns of aggregation. *Synedra investiens* cells were separated from one another and were lying in the ridges of the panels (Fig. 2B) whereas those of *S. tabulata* formed regularly arranged fan-shaped colonies (Fig. 2C). These colonies were seen to erect as the density of the community increased. *Achnanthes pseudogroenlandica* (Fig. 2D) and *Gomphonema kamtschaticum* were frequently observed on the panels. Both species were capable of forming a mucus peduncle, which in *G. kamtschaticum* could become ramified. Clump growth combined with the recruitment of new cells later produced complex assemblages, as shown on Fig. 2E−F. The spatial distribution of *A. b.* var. *parvula*, the dominant species in the intertidal zone, is shown in Fig. 4D. It did not show any preferred orientation or pattern. The cells were usually lying randomly among the detritus, the raphe side facing the substrate. This species was occasionally seen with a short stalk. In summary, these species of diatoms employ different strategies of spatial utilization.

## SETTLEMENT IN THE SUBTIDAL ZONE

The settlement of organisms on panels immersed subtidally (−5 m) at monthly intervals at Leggatt's Point and Pointe Mitis was examined. The first samples, taken 15−21 d after immersion, were always colonized by bacteria (Fig. 3A, B). The numbers were low initially and gradually increased with time. On all Pointe Mitis panels and on those immersed in August at Leggatt's Point the bacterial counts stabilized between $20 \times 10^4$ and $40 \times 10^4$ cells · mm$^{-2}$. High counts (e.g. $150 \times 10^4$ cells · mm$^{-2}$) were always followed by a sharp decrease. Diatoms always appeared later than bacteria on the plates, *C. costata* usually as the first species to be observed. The extent of time prior to diatom colonization ranged from 4 to 6 wk depending on whether the panels were immersed in July or in June (Table 6). The acceleration of diatom colonization later in the season was very pronounced at both Leggatt's Point and at Pointe Mitis. Panels immersed in July at this latter station showed colonization by *C. costata* after as little as 2 wk (column 4, Table 6). Although *C. costata* was the first species to settle its density remained low and only a slight increase during the season was observed. Highest numbers on subtidal panels were observed in July, when 364.5 cells · mm$^{-2}$ were counted.

*Synedra investiens* and *A. pusio* were other species observed at the beginning of the colonization period. The former species rarely reached densities sufficient to be quantified. *Amphora pusio* was observed simultaneously with *C. costata* at Pointe Mitis but occurred later at Leggatt's Point. *Amphora pusio* showed very characteristic growth patterns on all panels immersed throughout season. Initially, there was a latent phase of about 5 wk during which the number of cells fluctuated but remained below 100 cells · mm$^{-2}$. This was followed by a growth phase at the end of September, when the population reached 236−675 cells · mm$^{-2}$.

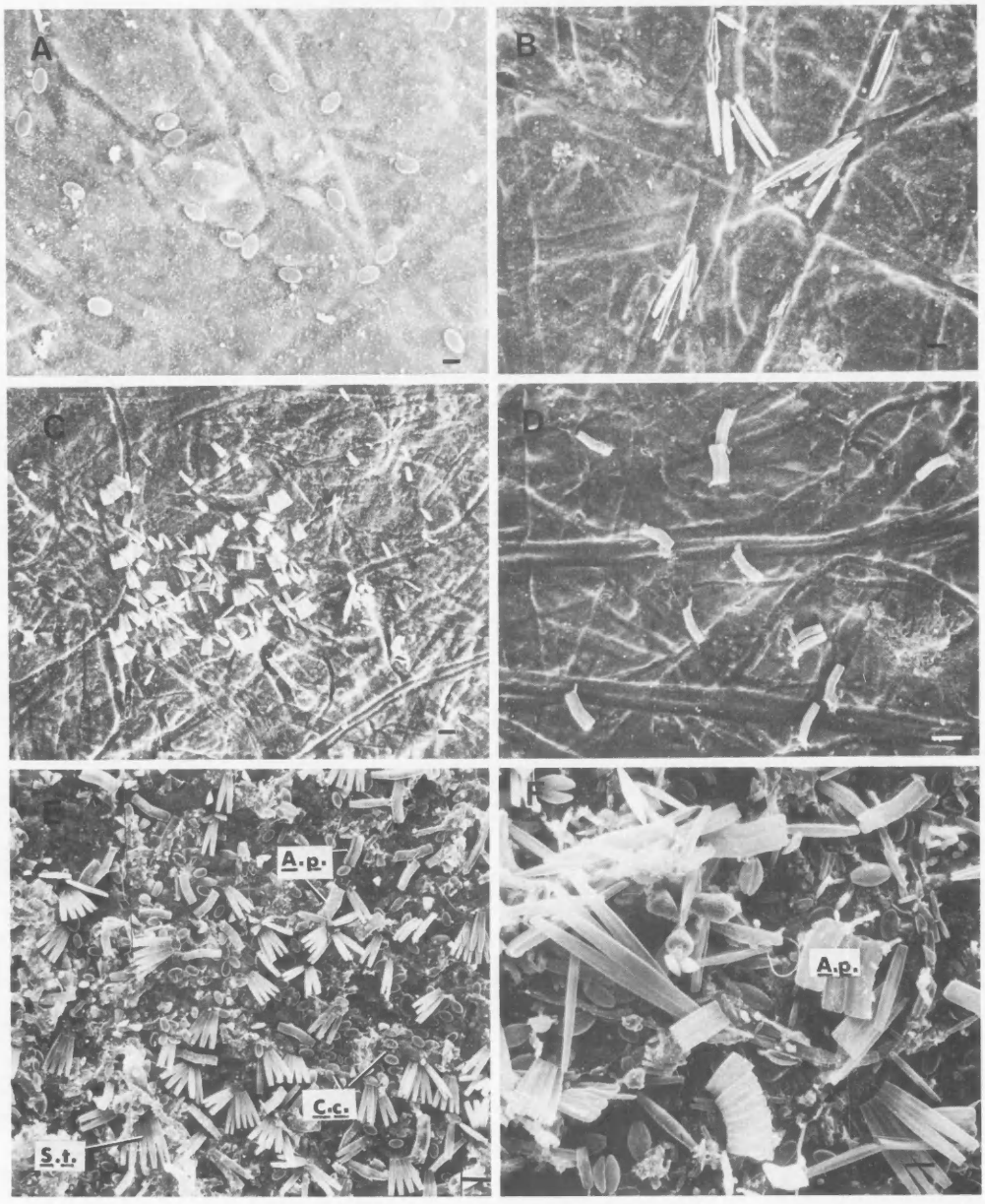


FIG. 2. Monospecific aggregates of dominant diatom species found subtidally. All micrographs were taken from the same sample. (A) *Cocconeis costata* (*C.c.*) laying with the raphe-bearing valve facing the substrate. (B) *Synedra investiens* cells laying horizontally. (C) Erect fan-shaped colonies of *Synedra tabulata* (*S.t.*). (D) *Achnanthes pseudogroenlandica* (*A.p.*) cells bearing a short peduncle. (E) The subtidal community as observed at −5 m at the end of September. (A−E) Scale bar indicates 100 μm. (F) Assemblage of the dominant diatom species found subtidally. Note how peduncle permits the cells of *A. pseudogroenlandica* (*A.p.*) to escape smothering. Scale bar indicates 10 μm.

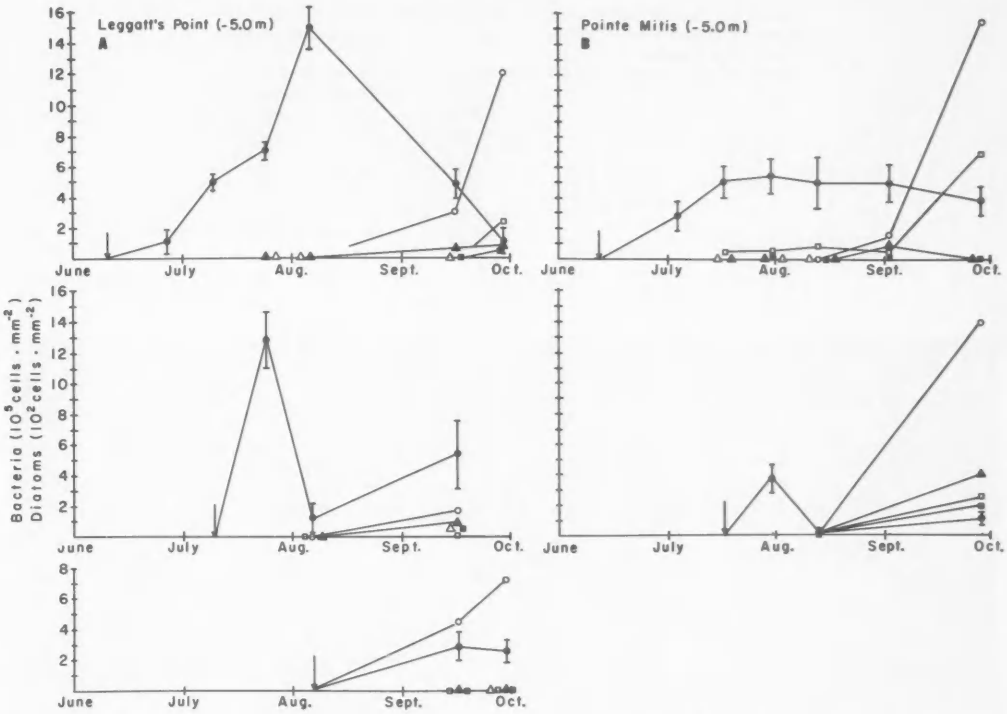


FIG. 3. Variations of abundance of bacteria and diatoms on five panels immersed at −5.0-m depth at Leggatt's Point (A) and Pointe Mitis (B) stations. The date of immersion of a panel is indicated by an arrow. Bacteria (♦), and the diatoms *Cocconeis costata* (▲), *Synedra investiens* (△), *Achnanthes pseudogroenlandica* (■), *Amphora pusio* (□), and *Synedra tabulata* (○). Values on the abscissa indicate the presence of less than 20 cells · mm$^{-2}$. Vertical lines represent the confidence intervals. Scale bars indicate 10 μm (A, B, D, F) or 100 μm (C).

Settlement of *C. costata*, *S. investiens*, and *A. pusio* was usually followed in August by colonization of *Synedra tabulata*. The density of this species remained under 100 cells · mm$^{-2}$ during the whole summer but, at the end of September, cell density reached 641−1485 cells · mm$^{-2}$ and the species completely covered the other species present (Fig. 3).

Other species (e.g. *A. pseudogroenlandica* and *G. kamtschaticum*) were frequently observed but their numbers remained low. Generally, on these subtidal panels numbers of diatom species increased as the season progressed, reaching a maximum number (between 15 and 20 depending on the panel) at the end of September. At first, detritus occurred as minute organic particles. Both the size and the quantity of the detrital particles gradually increased with time of immersion. The detritus was often associated with diatom patches, suggesting mechanical retention by the cells (Fig. 2E).

## SETTLEMENT IN THE INTERTIDAL AND SUBLITTORAL FRINGE

Intertidal panels placed between +1.0 and +2.5 m differed markedly from subtidal panels in both the species present and the pattern of colonization. After immersion in June, only detritus accumulated on panels for a period of 8 wk (Fig. 4A). Then, in early August bacteria appeared, 6 wk later than in the subtidal zone (Fig. 4B). Simultaneously, filamentous Ectocarpaceae began to grow and reached a maximum abundance 2 wk later (Fig. 4C). Thereafter, their abundance declined and the bacterial density increased until early September as the detritus continued to accumulate. *Achnanthes brevipes* var. *parvula*, the only species that showed important development on intertidal panels (Fig. 4D), was first seen in September. At first, cells occurred only in the depressions of the substrate, but later they spread wherever detritus accumulated, reaching densities as high as 2194 cells · mm$^{-2}$. No evident relationship could be found between the abundance of *A. b.* var. *parvula* and tidal height.

In view of the marked differences between subtidal and intertidal colonization, we decided to examine the bacterial and diatom settlement at intermediate levels varying from continuously immersed to semidiurnally emersed. At Les Ilets, three panels were placed at +0.3, +0.5, and +0.7 m above low water of spring tides on May 26, shortly after the spring tides, and were only emersed for short periods during each complete lunar cycle.

The fluctuations of the three major types of colonizers

TABLE 6. Time lag between immersion of panels and settlement of *Cocconeis costata* at different periods of the season at the (−5-m) Leggatt's Point and Point Mitis subtidal stations.

| Station | Date of immersion of plate | Time lag before colonization (wk) | Time between the first observation and an average of 65 ind./mm$^2$ (wk) |
|---|---|---|---|
| Leggatt's Point | June 10 | 6 | 7 |
| | July 9 | 4 | 3.5 |
| | August 6 | 3−5 | >13 |
| Point Mitis | June 10 | 5 | >22 |
| | July 14 | 4 | 2 |

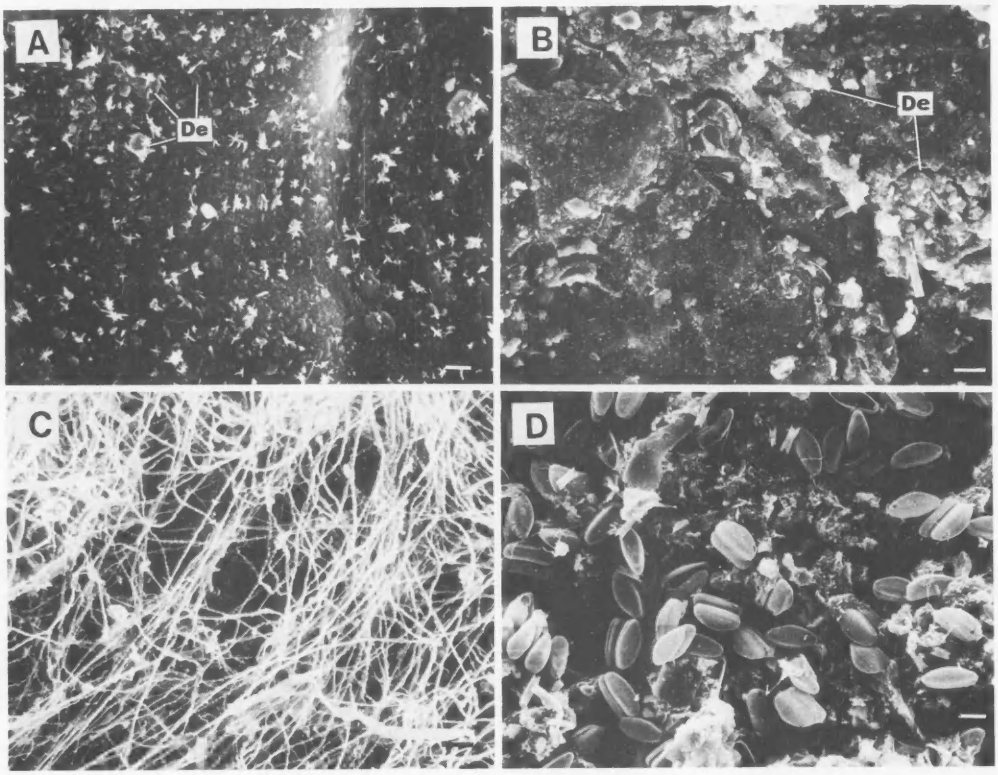


FIG. 4. Community development on a panel immersed in early June and semidiurnally exposed to air. (A) Detritus (De) accumulated after 6 wk (July 5) of immersion. (B) Detritus (De) and bacterial colonization after 10 wk (August 1) of immersion. (C) Dense filamentous Ectocarpaceae colonization after 12 wk (August 16) of immersion. (D) Colonization by *Achnanthes brevipes* var. *parvula* after 18 wk of immersion (September 26). Scale bars indicate 10 μm (A, B, D) or 100 μm (C).

(bacteria, diatoms, and filamentous Ectocarpaceae) on the panels are shown in Fig. 5. Bacteria were present on the panels throughout the summer at all three levels: they showed synchronous growth patterns with density peaks occurring in late July and the end of September. Their abundance remained about $10 \times 10^4$ to $20 \times 10^4$ cells · mm$^{-2}$, lower than the densities observed on the subtidally immersed plates. At all levels, diatoms were present on the panels at the first sampling date, 4 wk after immersion. The species were common subtidal forms (Table 3), *C. costata* and *S. investiens* achieving

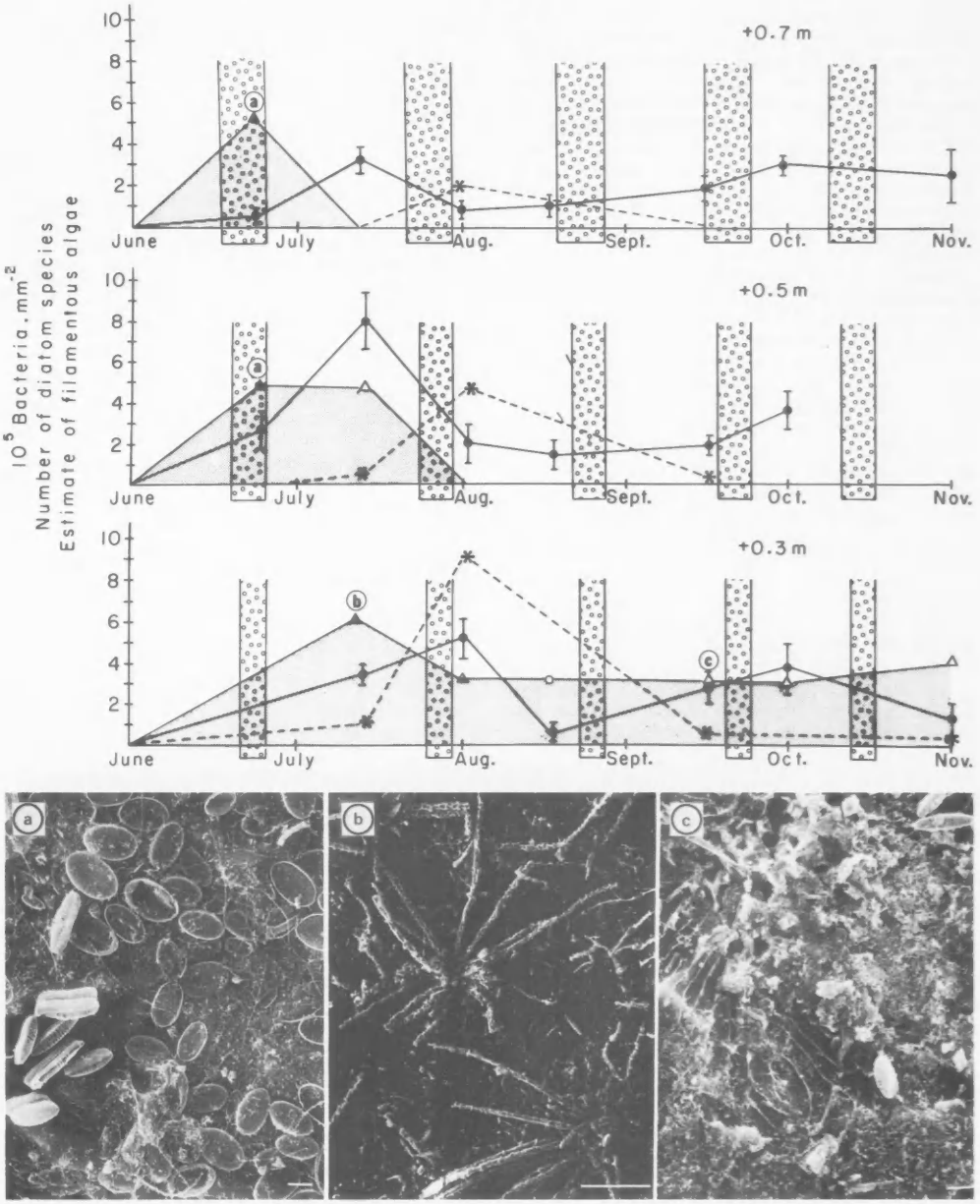


FIG. 5. Variation of abundance of bacteria (♦), filamentous brown algae (*), and number of diatom species (shaded area) on panels at station Les Ilets between June and November. Diatom abundance is not shown as reliable estimates could not be obtained at all times due to the presence of filamentous brown algae. The symbol associated to the number of diatom species indicates the dominant species: *Cocconeis costata* (▲), *Synedra investiens* (△), no dominance noted (○). Encircled letters refer to one of the micrographs below, illustrating the corresponding successionnal stage (see text). Scale bars indicate 10 μm (A, C) or 100 μm (B).

dominance. The former occurred in low densities at +0.7 m (56 cells · $mm^{-2}$) but reached a density of 1080 cells · $mm^{-2}$ at +0.5 m. It was dominant at both levels (+0.7, +0.5 m) sampled in mid-June. However, in June at both levels a large proportion of cells were damaged or dead and covered with bacteria (Fig. 5A). Because the fixation procedure was identical for all samples and bacteria were never observed on diatoms from subtidal samples nor from intertidal samples prior to the June sampling, these cells were presumed to be dead or in a poor physiological state. *Synedra investiens*, the other species frequently observed, reached only 47 cells · $mm^{-2}$.

Two weeks later, at mid-July, diatoms had completely disappeared from the +0.7-m level and had considerably diminished at the +0.5-m level, which was now dominated by *S. investiens*. In contrast, at the +0.3-m level, *C. costata* cells appeared in good condition and a density of 246 cells · $mm^{-2}$ was observed (Fig. 2A, 5A). Filamentous Ectocarpacae were first observed in mid-July growing in small clumps on the *Cocconeis*-covered substrate(Fig. 5B).

In early August, diatoms were no longer present at the +0.5-m level and were in low numbers at the +0.3-m level. Simultaneously, filamentous Ectocarpaceae had reached their maximum abundance and detritus was abundant. Later on, Ectocarpaceae decreased and they had disappeared by mid-September. Diatoms, especially *S. investiens*, occurred in low numbers until November at the +0.3-m level. Furthermore, during the autumn the cells appeared unhealthy (Fig. 5C).

At all stations subjected to emersion, filamentous Ectocarpaceae and detritus were abundant. Unfortunately these two components were difficult to evaluate quantitatively using the SEM. As an indication only, Fig. 6 compares the variations of the dry weight of total colonization in monthly (+0.3 m) and semidiurnally (+1.3 m) emersed conditions. There were significant variations of the dry weight at +0.3 m during the summer. The highest value ($122.5 \times 10^{-4}$ g · $cm^{-2}$) coincided with maximum growth of filamentous algae, which formed a 3-mm-thick olive-brown layer on the plates. Such a large change of biomass greatly overcomes the error involved in the diatom removal procedure. Colonization on the plates at the +1.3-m level was less conspicuous, more gradual, and led to the formation of a thin film in September. No confidence interval can be drawn for the values of the two curves in Fig. 6, as each point represents a single sample, made unavailable to SEM observation. Nevertheless, the curves give a good view of the general colonization patterns observed, contrasting the marked fluctuations at +0.3 m with the progressive growth at +1.3 m.

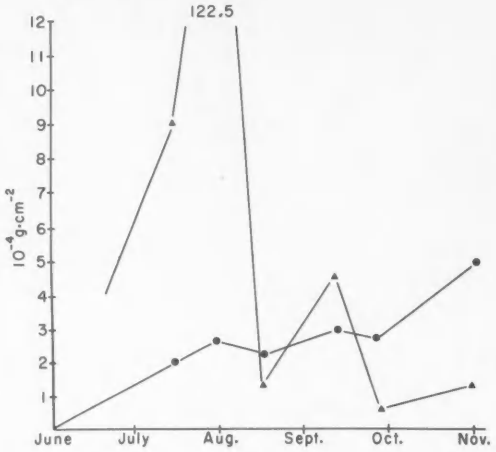


FIG. 6. Seasonal variation of the dry weight of material on panels emersed monthly (+0.3 m) at Les Ilets station (▲) and semidiurnally (+1.3 m) at Pointe Mitis (●).

## Discussion

Studies on diatom colonization of hard substrates using glass or Plexiglas panels and light microscopy are common. This technique provides an accurate view of species composition, abundance, and diversity of the assemblages (McIntire and Overton 1971; Bacon and Taylor 1976; Main and McIntire 1974; Moore and McIntire 1977). However, the technique has limitations as direct observations of species interrelations and of effect of sediment accumulation are not possible. An intermediate step to overcome surface scraping was achieved by Bélanger and Cardinal (1977), who observed undisturbed populations settled on glass slides using light microscopy. However, the three-dimensional aspect of community development could not be examined using this technique. Even with the SEM, which we have used, it is not easy to quantify interactions, but because we have documented the localization and orientation of the various taxa as well as the "smothering" growth pattern of *S. tabulata*, we feel confident about proposing some biological interactions. In the following discussion, in addition to examining the influence of physicochemical conditions, we attempt to explain how biological interactions might influence the structure of benthic diatom communities.

### Influence of Physicochemical Factors on Initial Settlement and Growth of Diatoms

In our study, the physicochemical factors may have influenced the time of settlement and abundance of many species. Rapid growth of bacterial population observed on panels immersed later in the summer can probably be explained by the summer increase of water temperature (Bott 1975). According to Cleve-Euler (1953), *A. pusio* is slightly halophilic. Thus, it was not surprising that the abundance of these species remained low during the summer while salinities were low (24−27‰), and only increased later in the summer as the salinity reached 28−29‰. The combination of temperature and salinity could also be of some importance. Optimal growth of *C. costata*, an arctic boreal species (Cleve-Euler 1953), was observed between 5 and 14°C and at 26−27‰ in the Gulf of St. Lawrence (Bélanger and Cardinal 1977). These preferences could explain the settlement of this species in early summer. Our results also suggest that high light intensities may influence the abundance of this species as the largest numbers were observed in shallow waters (e.g. station

Les Ilets). Emersion also was an important factor controlling the abundance of this species as it was not very common on the semidiurnally emersed plates and was eliminated from monthly emersed panels following their exposure at neap tides. The only species resistant to semidiurnal emersion, *A. b.* var. *parvula*, was commonly observed from February to March on partially exposed rocky surfaces (Hendey 1964) of the English coast. These conditions are very similar to those in which this species was observed in our study. The two other commonly observed species, *A. pseudogroenlandica* and *G. kamtschaticum*, are Arctic marine species (Boyer 1927 a, b; Hendey 1964) and, as expected, their maximum growth was observed in early November, when temperatures were lowest (2°C) and salinities maximum (28−29‰). The influence of tidal level on the hard substrate algal community has been investigated by many authors (Castenholz 1963; McIntire and Wulff 1969; Doty 1946) and our results are in agreement with previous observations. In our study, colonization was reduced at higher levels: the settlement of bacteria took up to 14 wk, the number of diatom taxa was reduced to a few tolerant species, and the abundance of Ectocarpacae was lower.

Bacterial colonization occurred earlier on continuously immersed subtidal panels than on intertidal panels. At increasing depth (>10 m in the Estuary) diatom growth decreased and became negligible. Similarly, shallow-water panels were colonized faster than deep-water panels. Comparison of panels immersed in July at 1-, 5-, and 10-m depths at Leggatt's Point (1 and 10 m, unpublished data) showed a time lag of 1, 4, and >7 wk, respectively, before diatom settlement. Here, perhaps higher light availability or water temperatures accelerated settlement, as temperatures were 12, 9, and 5°C, respectively, at these three depths. At 5-m depth, the time lag before diatom colonization also decreased from 6 to 4 wk on panels immersed in June (9−10°C) and August (15°C). Further studies are needed to document this aspect.

The low number of species enumerated in this study can be explained in many ways. First, it must be stressed that only the major species, occurring on at least 5% of the samples, were included in this study. There is also no doubt that a nonliving substrate is less favorable to diatom growth than macroalgae or marine angiosperms (Round 1971; Main and McIntire 1974). Furthermore, because the best growth depth for epiphytic diatoms is well above 5 m (Round 1961; Sladeckova 1962), we can assume that the communities described in this work were all limited to various extents by various physicochemical stresses, mainly high turbidity (low light intensity) and low temperature at 5-m depth and desiccation in the intertidal. The annual range of salinity for this area is 16−28‰ and could also partially explain the simplified community observed here. However the reduction of light to 1−5% at 5-m depth is the factor most likely to be limiting. All these factors combined result in a small species pool (Patrick 1967) and provide the best explanation for the simplified communities and interactions observed here.

The effect of grazing on attached diatom communities has been documented by many authors (Dickman 1968; Patrick 1972; Nicotri 1977), who showed the selective effect of predation. In our study, littorinid gastropods and sea urchins were occasionnally observed on the panels but the distinctively denuded "tracks" visible behind them were discarded from the sampling. The effect of grazing by amphipods and harparticoid copepods was investigated separately and this data will be published later. Thus, the communities described here are not only the result of biological interactions and physicochemical stress but also of grazing pressures.

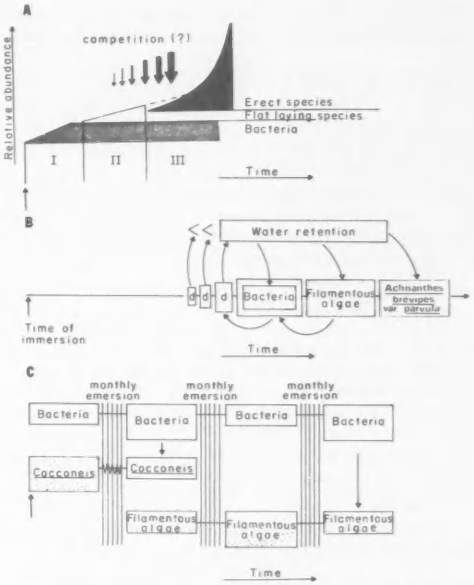


FIG. 7. Schematic representation of colonization in the subtidal (A), intertidal (B), and sublittoral fringe (C). Arrows indicate the various possible interactions between community components. The relative abundance of an organism is given by the size of the enclosure, and shaded ones indicate an eventual seasonal favorable factor; (d) detritus. Vertical lines refer to spring tide monthly emersion. Emersion disturbances are shown by a zigzag line.

In summary, the communities observed in this study can be characterized by their very slow development and low diversity.

## SUCCESSION IN THE LITTORAL ZONE

As indicated above, stations of similar level had similar and characteristic patterns of temporal succession. In the subtidal area, the pattern of colonization can be divided in three distinct phases, shown on Fig. 7A. During the first phase, only bacteria settled on the panels. As previously shown, the duration of this period varied with physicochemical factors. During the second phase, settlement of diatoms occurred and the cells remained grouped in separate clumps. Such simultaneous growth in separate clumps indicates that the presence of erect species is not conditioned by the earlier growth of flat-laying species, thus ruling out the idea of dependency toward the preceding colonizer currently used to identify "true succession." At first, the number of species and their abundance were low but they gradually increased with time. Detritus

TABLE 7. Summary of previous observations on sequences of diatom colonization.

| Observations | Substrate | Location | Authors |
|---|---|---|---|
| "Individuals of motile pennate diatoms which multiplied to form a film bound to the substratum by mucous secretions." "Those pennate diatoms... later on secrete gelatinous tubes inside which they arrange themselves and multiply as to give the appearance of branched filaments" | Panels and plates of glass, Plexiglas, vinyl acetate, wood, and iron | La Jolla, CA | Aleem (1957) |
| *Navicula* spp. followed by *Synedra* spp. and *Meridion* spp. | Glass slides | Prater's creek, SC | Reisen and Spencer (1970) |
| Bacteria followed by unicellular diatoms, then by *Ulothrix*, and filamentous and colonial diatoms (e.g. *Achnanthes* spp. *Navicula*, *Schizonema* spp.) and later on by *Ectocarpus* and *Enteromorpha* | Test plates treated with anti-fouling preparation | | Russell (1971) |
| A crust of *Cocconeis scutellum* (flat-laying species) was observed first, described "as an amorphous pavement of broken frustules and detritus (which) supports stalked and colonial diatoms. Non selective colonization by... ribbons of *Rhabdonema adriaticum*, stalked *Achnanthes longipes*, *Nitzschia* sp. (etc.) followed" | *Zostera marina* | Peetaquamscutt River, RI | Sieburth and Thomas (1973) |
| (1) Bacteria followed by (2) pennate diatoms and later on by (3) stalked diatoms | Wood | Fort Lauderdale, FL | Cundell and Mitchell (1977) |
| "At first, the community is a two dimensionnal one... When the growth becomes relatively heavy many of the species that formerly laid flat on the substrate now will stand upright because of jelly pads or their ability to produce jelly stalks. Thus a three dimensionnal community will be produced..." | ? | ? | Patrick (1977) |
| The last succession phases consisted always of long and spiny cells; at one station a bloom of many species started after the end of observations. At the two other stations, however, patches of *Enteromorpha* and blue-green algae were appearing at the end of experiments | Sediments, under laboratory conditions | | Admiraal (1977) |
| Unicellular forms of benthic algae like *Cocconeis costata* are followed later by *Cocconeis scutellum* and finally by *Fragilaria construens*, *Amphora* spp., and *Navicula* spp. | Glass slides | Gulf of St. Lawrence | Bélanger and Cardinal (1977) |
| "The first diatoms found attached were unicellular but after one or two months colonial forms were found..." | Rocky intertidal | Ria of Vigo (Northwestern Spain) | Niell (1979) |

accumulated in the diatom aggregations.

The third phase started when the clumps began to overlap following the multiplication and recruitment of new diatom cells. During this period the number of species and the cell density reached their peaks. Dominance by one species was soon observed and all the substrate available was occupied by diatoms which were partially covered by detritus. This period marked the beginning of potential species interaction such as competition for space (see Sieburth 1968; Patrick 1977). In the semidiurnally emersed intertidal zone, community development was very slow. Figure 7B illustrates the probable factors structuring the community in this zone. First, the detritus (d) progressively accumulated on the panels for many weeks before bacteria developed. Then, filamentous brown alga settled and grew extensively. Following their subsequent elimination, the substrate became covered with large amounts of detritus and bacteria. Diatoms then settled (only one tolerant species, *A. b.* var. *parvula*, was commonly observed) and their density increased slowly. Colonization by diatoms probably required the water retention provided by detritus (see Hopkins 1964), as in its absence no colonization was observed. This situation is the best example of "conditioning" observed in this study. The sublittoral fringe shares characteristics of both subtidal and intertidal zones. However, biological interactions are overshadowed by the monthly emersions. Figure 7C illustrates this pattern of colonization. As in the subtidal, bacteria and diatoms settled early and reached high numbers. At first, the species composition resembled that of the subtidal area, except for the presence of *A. b.* var. *parvula*, the dominant species of the intertidal zone. Following the first monthly emersion period, the community became similar to that of the intertidal one. Diatoms were reduced to a few tolerant species or were completely eliminated, and detritus became very abundant. Bacterial growth and detrital accumulation appeared concurrently with the decay of filamentous algae and diatoms. The maintenance of vestigial diatom populations at the lower (+0.3-m) level (station Les Ilets) may have resulted from the joint action of minimal shading and sufficient moisture retention by filamentous brown algae and detritus. Our observations suggest that the sublittoral fringe community was more perturbed by emersion than the semidiurnally emersed community.

### Are There Different Colonization Strategies for Benthic Diatoms?

For horizontally positioned species (*Cocconeis* spp., *Amphora* spp.), the ability to move using the raphe probably allows spacing of cells after multiplication, as well as movements to crevices of the substrate for increased protection. *Synedra tabulata* and *A. pseudogroenlandica* were observed to use all three spatial dimensions, thus allowing an increase in their density. The fact that these species appeared late in the succession when densities were high suggests that they are well adapted for colonizing in conditions of limited space, their mode of attachment allowing them to avoid the reduced illumination due to "smothering" by other species or detritus. Similar observations were made on *G. kamtschaticum*, a dendritic colonial form which achieved an almost complete independence of the substrate and reached dominance when overall densities were high (unpublished data). In fact, although these colonial forms are well adapted to occupy limited space and avoid smothering, they could have a detrimental effect on the lower canopy level by reducing available light and nutrients. However, because heterotrophy is common among benthic diatoms (Hellebust and Lewin 1970), further investigation is needed to confirm this possible detrimental effect. Such occupancy of the vertical dimension is probably achieved at the expense of community stability as adhesion to the substrate is limited and the dominant species become more exposed to grazing and wave conditions. If strategies of substrate occupation are of some ecological importance, a recurrent sequence of diatom colonization, eventually leading to the use of the vertical dimension, should be observed. Thus we suggest that dominance is most likely to shift in time from species laying flat on the substrate to progressively more erect species, except perhaps in high energy habitats where flat-laying species might remain dominant. Although the occurrence of stalked forms is apparently not dependent on the presence of flat-laying species (see Succession in the littoral zone section), the sequence of dominance always proceeded toward vertical species. A survey of the literature available (Table 7) also indicates that such a succession of strategies is indeed observed, no matter which type of substrate is examined. Furthermore, a qualitative description of this phenomenon was made by Patrick and Roberts (1979). Because similar vertical stratification has also been observed in kelp beds (Dayton 1975) and forests (Whittaker 1972), there probably is an analogous response to problems of space and light availability in all vegetal communities.

## Acknowledgments

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# Physical Control of Phytoplankton Production under Sea Ice (Manitounuk Sound, Hudson Bay)[1]

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LEGENDRE, L., R. G. INGRAM, AND M. POULIN. 1981. Physical control of phytoplankton production under sea ice (Manitounuk Sound, Hudson Bay). Can. J. Fish. Aquat. Sci. 38: 1385 – 1392.

In polar and subpolar seas, there are numerous accounts of phytoplankton blooms in the upper water column under the ice. Various mechanisms have been invoked to explain these blooms: the seeding of the underlying surface water by algal cells (epontic flora) released from the melting ice, the optimization of light utilization by the cells, and the stabilization of the upper water column by the low-salinity melting water. From studies conducted in Manitounuk Sound (Hudson Bay), it is proposed that phytoplankton blooms under the ice probably result from the simultaneous deepening of both the photic layer (seasonal light increase) and the stratified layer (low-salinity melting water). In ice-covered seas, the release of ice algae superimposes itself on the phytoplankton bloom, resulting in the observed algal increase under melting ice.

*Key words*: phytoplankton, under-ice blooms, ice flora, stability, nutrients, Hudson Bay

LEGENDRE, L., R. G. INGRAM, AND M. POULIN. 1981. Physical control of phytoplankton production under sea ice (Manitounuk Sound, Hudson Bay). Can. J. Fish. Aquat. Sci. 38: 1385 – 1392.

Dans les mers polaires et sub-polaires, on rapporte plusieurs cas de poussées phytoplanctoniques sous la glace, dans la partie supérieure de la colonne d'eau. Plusieurs mécanismes ont été proposés pour expliquer ces poussées : l'ensemencement des eaux de surface par des cellules végétales (flore épontique) libérées à la fonte de glace, l'optimisation de l'utilisation de la lumière par les algues ainsi que la stabilisation de la partie supérieure de la colonne d'eau par les eaux de fonte à faible salinité. Des travaux menés dans le détroit de Manitounuk (baie d'Hudson) permettent de proposer que les poussées de phytoplancton sous la glace sont probablement causées par l'enfoncement simultané de la couche photique (augmentation saisonnière de la lumière solaire) et aussi de la couche stratifiée (eaux de fonte à faible salinité). Dans les mers couvertes de glace, les algues qui s'écoulent de la glace s'ajoutent à celles qui croissent dans la colonne d'eau, ce qui cause l'augmentation de cellules observée sous la glace fondante.

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PHYSICAL control of phytoplankton production on continental shelves is a subject of growing interest among biological and physical oceanographers. After reviewing the literature on the subject, Legendre (1981) concluded that periodic stabilization and destabilization of the water column is the prime physical control of phytoplankton growth in productive shelf areas. Evidence of periodic phytoplankton blooms, either in the summer or in the winter, has been reported for the northwest European Shelf (Pingree et al. 1975, 1976, 1977, 1978) and the Scotian Shelf (Fournier et al. 1979), as well as for Puget Sound, a fjord of northwestern United States (Winter et al.

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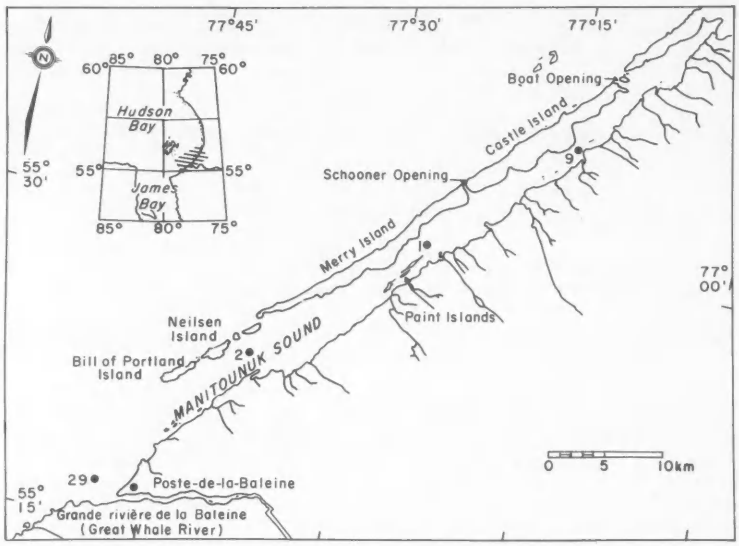


FIG. 1. Study area showing station positions. Manitounuk Sound extends from Bill of Portland Island to beyond Boat Opening.

1975), Saanich Inlet, western Canada (Takahashi et al. 1977), Auke Bay, Alaska (Iverson et al. 1974), and also for the St. Lawrence Estuary (Sinclair 1978; Fortier and Legendre 1979). In all these instances, the physical control of production is effected through the hydrodynamic regulation of both light (depth of the mixed layer versus critical depth) and nutrients (nutrient availability versus nutrient assimilation).

In polar and subpolar seas, there are numerous accounts of phytoplankton blooms in the upper water column, under the ice, in late winter and spring. These were reviewed by Horner (1976) and by Grainger (1977), who reported conflicting evidence as to the relationship between primary production in the water column and that in the overlying ice cover. Some of the reviewed papers suggest that the phytoplankton bloom in the water is directly initiated by the release of algal cells (epontic flora) from the melting ice whereas others claim that the release of cells to the underlying water is not a major source of the spring phytoplankton bloom. Meguro et al. (1967) explain the growth of Arctic diatoms as a function of solar radiation and the thickness of the sea ice: the cells can utilize daylight most efficiently if they grow in the sea ice early in the season, then move into the water column.

On the other hand, during the period of ice melt the surface salinity reaches its seasonal minimum. According to Allen (1971), "... low salinity has important consequences for phytoplankton ecology. Many of the diatoms which develop under the ice early in the season are killed by the low salinity, and the population does not fully recover until mixing restores the salinity of the surface waters." On the contrary, Saito and Taniguchi (1978) consider the melt water as a positive factor in initiating the under-ice bloom because at the same time as ice algae are released into the sea, a bloom occurs within the shallow surface layer which is (vertically) stabilized by the presence of low-salinity water. Consequently, two groups of phytoplankton then coexist in the surface water: the ice algae and the spring bloom diatoms. The relative importance of these trigger mechanisms for the spring bloom under the ice (seeding from the ice flora, light optimization, and water column stability) cannot be easily assessed because they all occur almost simultaneously in natural conditions at the time of ice melt.

Hudson Bay is a small mediterranean sea of the Atlantic Ocean, characterized by a $1.2 \times 10^6$ km$^2$ surface area and 128-m average depth. The drainage area of Hudson Bay, comprising some $4 \times 10^6$ km$^2$, provides a major source of freshwater to the Bay. Thus, the relative proportions and mixing characteristics of the continental freshwaters and the marine waters advected from the Atlantic Ocean determine the general characteristics of biological production in Hudson Bay. Manitounuk Sound (Fig. 1), located behind the Manitounuk Islands, is 60 km long by 3 km in average width, with depths ranging from 90 m at its entrance to 5–10 m at its inner termination. The Sound has a number of passages to Hudson Bay near its mouth but only two connections over its remaining length, these being Schooner Opening and Boat Passage. Only minor local sources of freshwater occur in the Sound. However, its water mass characteristics are greatly influenced by the intrusion of brackish water from the plume of the Great Whale River. In Manitounuk Sound, a permanent brackish layer exists under the ice, allowing discussion of possible trigger mechanisms of the under-ice bloom independently of water column stabilization from ice melt.

In the present work, the necessary conditions for an under-ice phytoplankton bloom in the protected and stratified Mani-

tounuk Sound are analyzed in order to discuss (i) the role of density stratification in initiating the spring bloom under the ice, (ii) its importance relative to the under-ice light intensity and seeding from ice algae, and (iii) how the presence of an ice cover modifies the basic hydrodynamic control of phytoplankton production encountered in more temperate shelf areas.

## Materials and Methods

Monthly sampling through the ice was conducted from mid-February through mid-May 1978, at four stations (Fig. 1): stn. 29 (27-m depth, not sampled in February), stn. 2 (50-m depth), stn. 1 (16-m depth), and stn. 9 (27-m depth).

Variables sampled to describe the physical environment included temperature, salinity, current velocity, local tide characteristics, meteorological information, and ice distribution and thickness. Temperature ($T$) and salinity ($S$) measurements were obtained using an Aanderaa current meter with a 0.5-min sampling rate. Currents were measured with similar instruments, either by profiling at discrete depths or by long-term moorings. Sampling rate for moorings was 5 min, excepting a 1-h rate for the 10-mo mooring at stn. 1. Recorded variables were temperature, conductivity, speed, current direction, and pressure.

All vector quantities were decomposed into along-shore ($U$) and offshore ($V$) components. Positive $U$ was directed along 250° mag. (230° true) while positive $V$ was 160° mag. The $U$ axis paralleled the local coastline and the major axis of Manitounuk Sound.

Water from 0, 5, and 10 m was collected through a 0.40-$m^2$ hole in the ice, using 1.7-L Niskin bottles and a small winch. Water samples were filtered and frozen for later determination of nutrients ($SiO_3$, $NO_2$ + $NO_3$, $PO_4$), using a Technicon autoanalyzer. Filters were frozen for determination of chlorophyll *a* and pheophytin *a* (fluorometric method). All the analyses followed the methods outlined in Strickland and Parsons (1972). Water samples were preserved with Lugol and formaldehyde, the Utermöhl (1931) method being used to enumerate the phytoplankton cells.

Three-centimetre-thick slices were cut at various levels in the 0.40-$m^2$ ice block and melted for later laboratory analysis. In regard to the present paper the analysis focuses on the algal species in the bottom layer of the ice. Samples were preserved in a similar manner as the phytoplankton and examined later under the microscope.

Access to the field stations was by helicopter from Poste-de-la-Baleine. In addition to the above, surface water at stn. 2 (still covered with broken ice) was sampled on two occasions from mid-June to mid-July 1978, using a helicopter.

## Results

Surface nutrient concentrations did not show a strong horizontal gradient within Manitounuk Sound or as compared with stn. 29 at the mouth of the Great Whale River (Fig. 2). Only the silicate appeared somewhat more abundant in the upper reaches of the Sound (stn. 1 and 9) than at the mouth (stn. 2 and 29). Furthermore, no major temporal changes in surface concentration were observed from February through

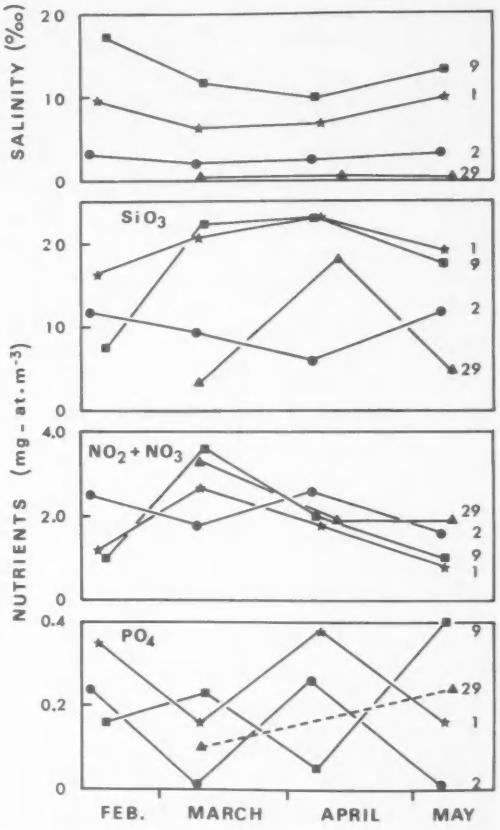


FIG. 2. Salinity and nutrients in the surface water (0 m) under the ice. No $PO_4$ data in April at station 29.

May. There was perhaps a slight decrease in nitrate, from March to May. However, there was a significant surface salinity gradient along the Sound, which persisted throughout the sampling period. This feature is clearly demonstrated in the salinity profiles taken through the ice during early May (Fig. 3).

The constancy of conditions in the lower layer can be seen in Fig. 4, in which along-channel velocity, temperature, and salinity at 10 m have been plotted for stn. 1 during the first 2 wk of May. Tidal currents varied from ~10 cm · $s^{-1}$ during neap conditions on days 122 and 123 (Julian) to ~25 cm · $s^{-1}$ for springs on days 129 and 130. Temperature (slightly above freezing) and salinity were essentially constant within the accuracy of the sensors. Cross-channel velocities (not plotted) were less than 5 cm · $s^{-1}$ throughout the record. Nutrients were also very constant at 10-m depth, with only a relatively small standard deviation about the mean for the four stations and the four sampling months taken together: 9.6 ± 1.6 mg-at $SiO_3$-Si · $m^{-3}$, 2.8 ± 1.2 mg-at ($NO_2$ + $NO_3$)-N · $m^{-3}$, and

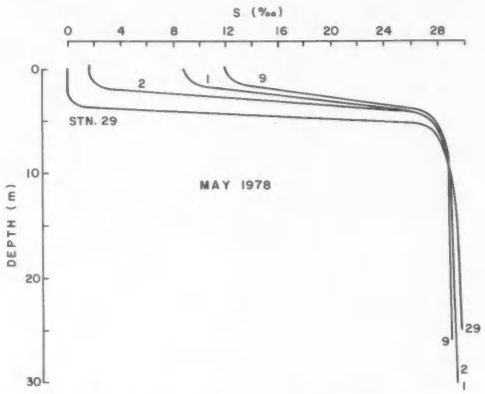


FIG. 3. Vertical profiles of salinity in May under a 1- to 1.5-m ice cover.

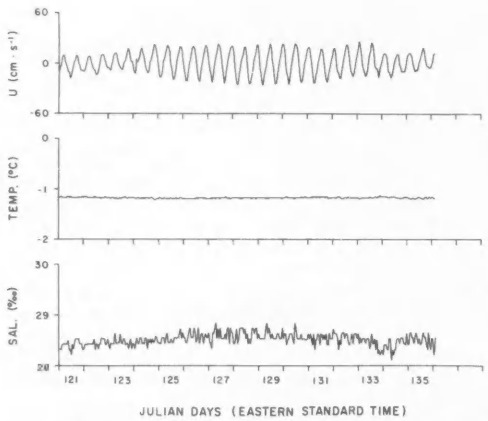


FIG. 4. Time series of $U$ (along-channel velocity), temperature, and salinity for station 1 at a depth of 10 m. Horizontal axis in Julian days (121: May 1, 1978).

$0.86 \pm 0.25$ mg-at $PO_4$-P $\cdot$ $m^{-3}$. Except for silicate, the deeper waters were richer in nutrients than the brackish surface water. The sampling was completed before the beginning of the spring runoff, the discharge of the Great Whale River being lower than 300 $m^3 \cdot s^{-1}$ on May 10, 1978. Comparative values during spring runoff exceed 700 $m^3 \cdot s^{-1}$. All values were taken some 55 km upstream of the river mouth by the Ministère des Richesses naturelles du Québec.

Chlorophyll concentrations in the surface water (0 m) under the ice (Fig. 5) remained very low through April, with the exception of stn. 29, the average being $0.08 \pm 0.03$ mg $\cdot$ $m^{-3}$ (stn. 29 in April excluded). There was a dramatic increase in chlorophyll during May, still under full ice cover. At 5 and 10 m, chlorophyll remained very low from February through May, with an average concentration of $0.04 \pm 0.02$

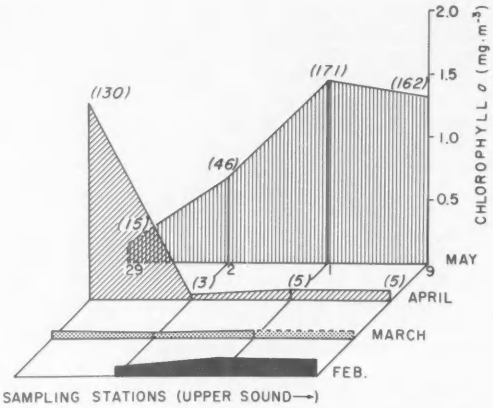


FIG. 5. Under-ice changes of chlorophyll in the surface water of Manitounuk Sound. No surface value was recorded at station 9 in March. Figures in parentheses correspond to $10^6$ cells $\cdot$ $m^{-3}$ in April and May (empty cells excluded).

mg $\cdot$ $m^{-3}$, in contrast with the increase found in the surface waters. Average pheophytin $a$ was $0.25 \pm 0.09$ mg $\cdot$ $m^{-3}$ at 0 m and $0.06 \pm 0.02$ mg $\cdot$ $m^{-3}$ at 5 and 10 m, which was higher than the chlorophyll concentrations observed under the ice, except in the surface water near the end of the ice-covered period (Fig. 5).

Chlorophyll $a$ concentrations in April and May were paralleled (Fig. 5 and Table 1) by the total number of phytoplankton cells (excluding the empty cells) in the surface water. The chlorophyll increase in April at the mouth of Great Whale River (stn. 29) was essentially made up of flagellates whereas the May increase corresponded to an increase in both the number of cells and in the number of taxa other than flagellates. Most of the pennate diatoms observed in the surface water were simultaneously present in the bottom layer of the overlying sea ice (Table 1). In contrast, all the centric diatoms (with a single exception) and all the dinoflagellates in the water were not simultaneously observed in the ice flora. Dinoflagellates were very scarce both in April and May.

Figure 6 shows an inverse relationship between surface chlorophyll and both the density difference in the 5-m surface layer and the nitrate concentration at 0 m in May. A relationship was found between the relative abundance of the phytoplankton groups and the salinity in the surface layer during April and May (Fig. 7): the proportion of flagellates decreased with increasing salinity whereas the proportion of both pennate and centric diatoms increased. The Spearman's rank correlation coefficient between salinity and the proportion of flagellates ($\rho = -0.643$) or pennate diatoms ($\rho = 0.619$) was significantly different from zero at the 5% probability level whereas that between salinity and the proportion of centric diatoms ($\rho = 0.393$) was not.

Due to sampling problems at the time of broken ice (surface water collected from a helicopter), only a few data were available under these conditions. From mid-June to mid-July, data for stn. 2 (Table 2) show that a very drastic change occurs at the time of ice breakup, at least in the surface waters.

TABLE 1. Phytoplankton taxa observed in April (A) and May (M) in the surface water under the ice. Numbers ($10^6$ cells · $m^{-3}$) excluding the empty cells; +, less than $0.5 \times 10^6$ cells · $m^{-3}$; −, taxon not observed; †, taxon simultaneously observed in the overlying sea ice.

| Taxa | Stn. 29 | | Stn. 2 | | Stn. 1 | | Stn. 9 | |
|---|---|---|---|---|---|---|---|---|
| | A | M | A | M | A | M | A | M |
| Flagellates | 130 | 13 | 3 | 44 | 3 | 71 | 4† | 102† |
| Chrysophyta: Bacillariophyceae | | | | | | | | |
| Centricae | | | | | | | | |
| *Chaetoceros karianus* | − | − | + | − | + | 36 | + | 21 |
| *Chaetoceros septentrionalis* | − | − | − | − | − | + | − | 1† |
| *Cycloteila* spp. | − | − | − | − | − | − | − | + |
| *Melosira nummuloïdes* | − | − | − | − | − | 2 | − | − |
| Unidentified | − | + | − | − | − | 1 | − | − |
| Pennatae | | | | | | | | |
| *Achnanthes taeniata* (?) | − | − | − | − | − | − | − | + |
| *Amphiprora* spp. | − | − | − | − | − | − | +† | +† |
| *Fragilaria cylindrus* | − | − | − | − | 1† | 15† | +† | 5† |
| *Navicula oestrupi* | − | − | − | − | − | 1† | − | − |
| *Navicula septentrionalis* | − | − | − | − | − | 2† | − | − |
| *Navicula vanhoeffenii* | − | − | − | 1† | 1† | − | − | 5† |
| *Navicula* spp. | − | + | + | − | +† | 23† | 1† | 24† |
| *Nitzschia closterium* | − | − | − | − | − | − | − | 1† |
| *Nitzschia frigida* | − | − | − | − | − | 16† | − | +† |
| *Nitzschia* spp. | − | − | − | − | +† | +† | +† | 1† |
| *Pinnularia quadratarea* var. *bicontracta* | + | − | − | − | − | +† | − | − |
| Unidentified | − | 1 | − | + | − | 1 | − | − |
| Pyrrophyta: Dinophyceae | | | | | | | | |
| *Gonyaulax* spp. | − | − | − | 1 | − | 2 | − | 1 |
| Unidentified | − | − | − | − | − | − | + | − |
| Total | 130 | 15 | 3 | 46 | 5 | 171 | 5 | 162 |

Nitrate concentrations drop to a very low level while phosphates tend to increase; only silicate concentrations remain uniformly abundant.

## Discussion

### PHYSICAL CONDITIONS AND PHYTOPLANKTON

In contrast with late summer conditions, when the open water surface plume of the Great Whale River covered an area of some 50−100 $km^2$ near the river mouth, the presence of an ice cover on Hudson Bay and Manitounuk Sound resulted in a marked increase of the plume area (~1000 $km^2$) during the winter−spring period (Ingram 1981). The influence of this brackish layer adjoining the ice extended over the complete length of the Sound, providing salinities of less than 4‰ at stn. 2 in May 1978. Modification of the upper layer (typically 5 m in depth during May) was a gradual process because of the marked stability of the pycnocline separating it from the ambient waters underneath. Calculated gradient Richardson numbers over the pycnocline in May ranged from 20 to 50, typically an order of magnitude greater than values obtained under ice-free conditions in the previous summer. Richardson numbers compare the stabilizing effect of buoyancy to the destabilizing influence of vertical shear in the horizontal velocity field over a given depth interval. Smaller numbers are taken to indicate a greater tendency for vertical mixing (Turner 1973). The values calculated above were obtained from single current meter profiles. The increase in surface water salinity from the mouth to end of the Sound results from vertical entrainment and mixing of salt into this layer, thus providing higher salinities with increasing distance from the plume source. Thus a very stable two-layer system is found under the ice, extending from the mouth of Great Whale River (stn. 29) to the end of Manitounuk Sound. In May (Fig. 4), $T-S$ characteristics in the lower layer were quite constant. Although not shown, $T-S$ values in the lower layer during the February−April period were also relatively constant along the axis of the Sound.

Prior to May, salinity and nutrient values in the under-ice surface layer (Fig. 2) remained quite constant. At the same time (with the exception of stn. 29 in April) chlorophyll concentrations were very low (Fig. 5) and pheophytin high relative to the chlorophyll. This is an indication of phytoplankton cells in poor physiological condition. As the presence of a very stable water column and high nutrients indicate typical spring bloom conditions, our results suggest that solar energy under the ice was too low for any significant phytoplankton growth at this time.

Towards the end of the ice-covered period, there was a sharp rise in chlorophyll under the ice (Fig. 5). In May, lower nitrate concentrations (Fig. 2 and 6b) and the inverse relationship between chlorophyll and nitrate (Fig. 6b) both suggest an active assimilation of nutrients by the phytoplankton. This might eventually exhaust the limiting nutrient in the surface layer. Low nitrate concentrations, observed at stn. 2 following the breakup (Table 2), may be the result of such an exhaustion, which indicates the limiting potential of nitrogen

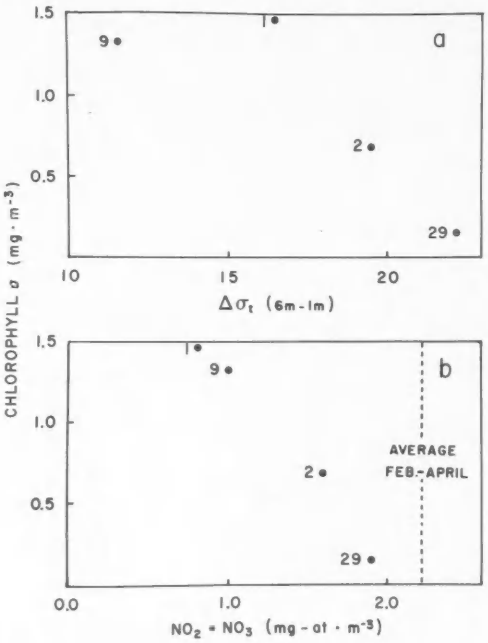


FIG. 6. Relationship between surface chlorophyll and (a) density stratification between 1 and 6 m and (b) nitrate for ice-covered conditions in May. Station numbers are shown.

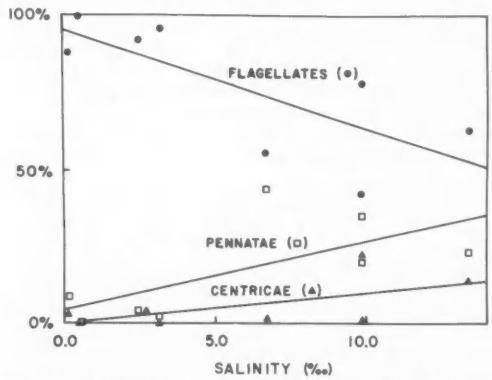


FIG. 7. Relative abundance of surface flagellates and diatoms (pennate and centric) as a function of surface salinity under ice in April and May 1978.

in Manitounuk Sound, as discussed by Legendre and Simard (1979) for summer conditions. Moreover, a strongly stratified two-layer system cannot sustain growing phytoplankton for very long because high stability prevents the replenishment of the surface layer nutrients from the deeper waters. Any vertical advection of underlying water is therefore an advantage for sustained phytoplankton growth. Increasing surface salinity with increasing distance from the plume source (Great Whale River) is an indication of such a vertical advection and it explains the general increase of chlorophyll with decreasing stratification of the surface layer (Fig. 6a) observed along Manitounuk Sound.

TABLE 2. Mean (standard deviation) of nutrients sampled in the surface waters (0 m) of station 2 under the ice cover (February through May) and in broken ice conditions (mid-June to mid-July).

| | Concentration (mg-at · $m^{-3}$) | |
|---|---|---|
| Nutrient | Feb.–May | June–July |
| $SiO_3$ | 9.8 (2.7) | 7.5 (1.2) |
| $NO_2 + NO_3$ | 2.1 (0.5) | 0.1 (0.1) |
| $PO_4$ | < 0.2 | 0.49 (0.17) |

The chlorophyll increases are paralleled by the numbers of cells (Fig. 5). Most of the pennate diatoms in the surface water are likely of ice origin as they are simultaneously present in both the ice and the water (Table 1). It is not possible, without autoradiographic studies, to establish if the pennate diatoms in the water are active. On the contrary, centric diatoms and also the few dinoflagellates in the water were probably not released from the ice as they were not simultaneously found in the ice samples. According to Saito and Taniguchi (1978), during late winter and early spring the surface phytoplankton community is occupied by both ice species and spring bloom diatoms, which corresponds to findings in Manitounuk Sound. The low pheophytin concentration relative to chlorophyll in May and the inverse chlorophyll to nitrate relationship (Fig. 6b) indicate that some phytoplankton cells were actively growing in the under-ice surface water. The percentage increase of both centric and pennate diatoms with salinity (Fig. 7) does not support the hypothesis of a simple drainage of ice algae into the underlying surface waters. In the highly stratified waters of Manitounuk Sound, the ultimate factor of the under-ice bloom appeared to be the seasonal increase in solar energy, which simultaneously drives an increase of phytoplankton production in the water column and initiates a slow melting of the ice cover, with concomitant drainage of ice algae into the underlying water.

The chlorophyll increase at the mouth of the Great Whale River (stn. 29), in April, corresponds to a flagellate bloom (Table 1) and it might be related to phenomena occurring in the River itself. These do not obviously affect the three other stations in the Sound.

## LIGHT AND HYDRODYNAMICS

The average daily solar radiation at Inoucdjouac (about 330 km north of Manitounuk Sound) was approximately the same in April (20.1 MJ · $m^{-2}$) and in May (20.4 MJ · $m^{-2}$) 1978. Similarly, the ice thickness at the four stations remained unchanged from April to May. However, following the increase in air temperature (from −8°C in April to +1°C in May), the ice was almost snow-free in May, thus allowing a deeper penetration of light in the water under the ice. In Frobisher Bay, Canadian Arctic, Grainger (1977) reports that the most efficient phytoplankton production occurs under snow-free ice.

Some of the phytoplankton blooms reported in the literature have been related to the physical conditions prevailing under

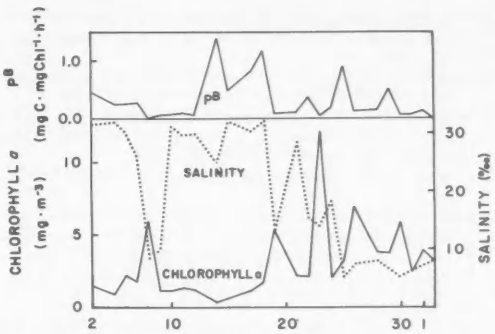


FIG. 8. Under-ice surface salinity, chlorophyll, and photosynthetic potential ($P^B$: under 700 lx illumination) in June and at the beginning of July at Point Barrow, Alaska. Data from Horner (1972, Fig. 4 and 5).

the ice. At Point Barrow, Alaska, for instance, peaks of chlorophyll under the ice were associated with salinity minima (Fig. 8). Furthermore, low salinities at the end of the ice melt did not preclude occurrences of relatively high photosynthetic potential ($P^B$: production per unit chlorophyll *a*, here, under an artificial illumination of 700 lx).

Previous studies for various Arctic environments (see the introduction) associate the initiation of the bloom with the melting of the ice, stressing the role of ice algae thus released into the water. However, observations in Frobisher Bay, Canadian Arctic (Grainger 1977), show a time lag between the addition of plant material from the sea ice and the first increase in phytoplankton. Furthermore, many authors maintain that the release of cells is not a major source of the phytoplankton bloom (see the introduction). It may be, therefore, that the critical role of stratification was underrated in many studies as increased stratification and release of ice algae occur simultaneously at the time of melting. On the other hand, results from Manitounuk Sound demonstrate that a stabilized water column is not enough to trigger an under-ice bloom, in absence of adequate illumination. It appears that increasing light intensity under the ice and stable stratification act as a dual trigger mechanism of blooms under the ice. Because ice algae are released in the water column at about the same time as the spring bloom in the surface water, the resulting phytoplankton community is made of both ice and spring bloom species (Saito and Taniguchi 1978). In some instances, the melting (with concomitant release of ice algae) may well not lead to a bloom, in which cases the only algae increasing under the ice would be the ice species. Many discussions about the role of ice algae in the blooms under ice may be related to the time of the sampling relative to the melting–stratification–bloom sequence or to instances without an actual bloom.

In temperate waters, the spring bloom results from the seasonal deepening of the photic layer and shallowing of the stratified layer, the phytoplankton outburst occurring when the shallowing depth of the surface layer reaches the deepening critical depth (Riley 1942; Sverdrup 1953). In Manitounuk Sound, on the contrary, the phytoplankton bloom proceeds only from the deepening of the photic layer as the persistent low-density layer of brackish water creates conditions of constant stratification under the ice. For cases of an unstratified water column underlying the ice, the bloom presumably results from the simultaneous deepening of both the photic and the stratified layers, at the time of ice melting. This concurrent deepening of the two layers is probably critical for the first stages of the bloom as phytoplankton cells may then easily be lost from the very thin photic layer. In ice-covered seas, the release of ice algae superimposes itself on the phytoplankton bloom, with or without a time lag, resulting in an increase of algal cells under melting ice.

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# Production Responses for Multi-species Fisheries

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AGNELLO, R. J., AND L. G. ANDERSON. 1981. Production responses for multi-species fisheries. Can. J. Fish. Aquat. Sci. 38: 1393–1404.

Production equations are estimated for five major species of fish harvested in the Northwest Atlantic including Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), flounder (*Pleuronectiformes*), redfish (*Sebastes marinus*), and herring (*Clupea harengus*) using data collected by the International Commission for the Northeast Atlantic Fisheries from 1960 to 1974. A log-linear regression specification is used in which the relationship between catch of a vessel and several factors including days fished and vessel characteristics is estimated simultaneously. Vessel characteristics are represented as (0,1) categorical variables, and include a variable indicating the target species designated by the caption. Various settings for the target species variables allow the estimated equation to represent either a by-catch or target catch equation. The production parameters estimated by the regressions are used to analyze the effects of current quotas set by the New England Fisheries Management Council. For some fleets we find a likelihood of idle capacity given the current quotas.

*Key words*: fisheries economics, multi-species harvesting, Northwest Atlantic fisheries

AGNELLO, R. J., AND L. G. ANDERSON. 1981. Production responses for multi-species fisheries. Can. J. Fish. Aquat. Sci. 38: 1393–1404.

Les données recueillies par la Commission internationale des pêcheries de l'Atlantique nord-ouest entre 1960 et 1974 ont été utilisées pour estimer les équations de production de cinq principales espèces de poissons récoltées dans l'Atlantique nord-ouest, la morue de l'Atlantique (*Gadus morhua*), l'aiglefin (*Melanogrammus aeglefinus*), les plies (*Pleuronectiformes*), le sébaste (*Sebastes marinus*) et le hareng (*Clupea harengus*). Nous avons fait appel à une régression log-linéaire dans laquelle la relation entre les prises d'un bateau et plusieurs facteurs, y compris jours de pêche et caractéristiques du bateau, est estimée simultanément. Les caractéristiques des bateaux sont représentées comme (0, 1) variables catégoriques et comprennent une variable qui indique l'espèce cible, telle que désignée dans la légende. Divers arrangements des variables d'espèces cibles font que l'équation estimée peut être soit une équation de prises fortuites, soit une de prises cibles. Nous avons utilisé les paramètres de production estimés par les régressions pour analyser les effets des contingents présentement établis par le *New England Fisheries Management Council*. Dans le cas de certaines flottilles, nous constatons qu'avec les contingents actuels, il est probable qu'il y ait surcapacité.

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IN this paper we describe results of a study to estimate production responses for individual fishing vessels where several species are harvested jointly. The paper extends the existing literature on the estimation of fishery production functions by explicitly differentiating direct (target) catch from incidental catch (by-catch) in a production model. By-catch is formally defined as catch of a fish species by vessels other than those indicating that species as a target whereas directed catch is the harvest by vessels declaring those species to be a target. By-catch for many fisheries is significant and thus must be managed to comply with extended jurisdiction laws such as the U.S. Fisheries Conservation and Management Act of 1976.

The estimation of fisheries yield or production functions is oftentimes complicated by a lack of data, externalities, economics of scale, and joint production. In addition, the problems are increased by the difficulty in defining the unit of observation, the ultimate inputs, and in correctly analyzing the renewable nature of the living capital, especially with regard to its nonappropriability by the individual firms in the industry.

Related to the unit of observation is the problem of defining exactly what constitutes the effort that is actually applied. Different vessels (1) use different types of gear, (2) are used for fishing at different times and locations, (3) are manned by

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crews with different skill levels, etc. And even if this indexing problem can be solved in a satisfactory manner, it is difficult to assess exactly what effect effort will have on the stock. Two reasons for this last difficulty are potential biological and technological interdependencies between different stocks of fish (see Anderson 1975). Biological interdependence occurs when the size of two or more stocks are significantly related to each other through the ecological system (i.e. they are competitors for some facet of the environment or they have a predator/prey relationship), and so effort directed at one species will affect its size and also the size of other related stocks. Technological interdependencies occur when effort directed at one species will also yield a by-catch of one or more other species. This relationship is difficult to specify because in some instances the amount and makeup of by-catch is truly incidental while in others they can be modified by various fishing strategies. When the latter is true, we have come the full circle and are back to the problem of defining effort.

Most research on the estimation of fisheries production functions has been done by fisheries biologists using standard biological handbooks such as Gulland (1969), Ricker (1975), and Royce (1972). This approach uses fishing mortality as the basic unit of measure. That is, biologists study how fishing mortality and its relation to natural mortality affect the size of the catch and of the stock. Examples of such work can be found in Beverton and Holt (1957), Dickie (1962), Fox (1975), Gulland (1974), Larkin (1973), and Schaefer (1957).

Economists on the other hand are normally concerned with specifying the relationship between fishing effort and fishing mortality, and between the economic inputs used in the fishery and the actual amount of fishing effort applied to the stock. Examples of economic fisheries analyses based firmly on biological models include Agnello and Anderson (1977), Bell (1972), Bell and Fullenbaum (1973), Dow et al. (1975), Gates and Norton (1974), Griffin et al. (1976), Hannesson (1975), Leung and Wang (1976), O'Rourke (1971), and Spence (1973). Another economic approach proceeds by modifying economic production models to fit biological problems rather than adapting biological models to include economic issues. This approach is taken by Huang and Lee (1976), and Carlson (1973). The empirical work done so far has been successful enough to encourage further attempts in this direction.

In this paper we take the approach of modifying an economic production function model to estimate short-run production responses using data from the Northwest Atlantic. Whereas our approach will not be able to address all of the issues discussed above, it was formulated, tested, and interpreted with them in mind. We abandon the industry-model approach in favour of individual observations so that the relative efficiency of different types of vessels can be dealt with directly. An important new feature of this paper is the use of a model which allows yield functions and relative efficiencies of vessels to be jointly estimated. Within this framework we also distinguish between directed catch and by-catch.

Econometric issues such as separability of the production function, full information estimation of multiple outputs, jointness of technology, and simultaneity bias, while important, are not the main focus of attention in this paper. There are two reasons for a less general approach than might be desirable from an econometric viewpoint. First, we simply do not have the factor price, output price, and cost information corresponding to the vessels in our data set which would allow us to investigate some interesting econometric issues. If appropriate factor and product price information were available, the most general approach to production function estimation is to estimate cost and factor demand or share functions. Diewart (1973) and Christensen et al. (1971) propose such functions and Hall (1973) relates the analysis to multiple outputs. Once cost or factor demand functions have been estimated, the principles of duality allow the parameters of the production function to be recovered uniquely. The principles of duality were derived by Shephard (1953) and Uzawa (1964), and form the theoretical basis for much of the current econometric work on production analysis. Secondly, our pragmatic approach provides a great deal of useful information for fishery managers in distinguishing between directed catch and by-catch as well as efficiencies of various vessels without the complexity of a more general econometric treatment relying on production duality theory.

## Model and Data

When estimating fishery production functions, specification of both outputs and inputs presents a complex problem. For a multiple output fishery, there is no basis for allocating inputs and outputs. In addition, since the production parameters and the value of outputs generally differ among species, aggregation (whether by weight, value, or something else) must proceed cautiously. To the fishery manager, aggregation, however performed, may be undesirable because the catch of individual species is usually regulated. Since recruitment, growth, and natural mortality may differ across species, separate yield functions exist for each species. The policymaker must therefore formulate harvest policies appropriate for individual species and requires production information specific to individual species to accomplish this task.

Consideration of multi-species outputs of vessels is necessary for most international fisheries where by-catch is large. Table 1 shows directed catch and by-catch for selected species harvested off the U.S. New England coast from 1960 to 1974, including Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), flounder (*Pleuronectiformes*), redfish or ocean perch (*Sebastes marinus*), and herring (*Clupea harengus*). For these species by-catch generally exceeds directed catch.

In specifying inputs the traditional approach of fishery biologists and economists is to combine inputs of heterogeneous vessels using some weighting method. This is done either to obtain a measure of aggregate effort to estimate an industry production function or to obtain comparable measures of vessel effort to estimate a micro (vessel) production function. In a multi-species fishery where by-catch is of significant magnitude relative to directed catch, aggregation is complicated further by the jointness of production. It is unclear which vessels should be included in the definition of the industry. Several industries (classified by directed species) exert pressure on the resource stock of a single species, and the production coefficients may vary for directed and by-catch.

TABLE 1. Directed catch and by-catch for Georges Bank and Gulf of Maine, 1960–74 (in thousand metric tonnes).[a]

| Species | Directed catch | By-catch |
|---|---|---|
| Cod | 150 | 374 |
| Haddock | 268 | 470 |
| Flounder[b] | 340 | 135 |
| Redfish | 84 | 114 |
| Herring | 1328 | 1733 |

[a]Source: *Statistical Bulletin*, Vol. 24, 1974, International Commission for the Northwest Atlantic Fisheries.

[b]Flounder includes all types.

In this paper we employ a pragmatic economic approach to fishery production function estimation which is primarily intended as a predictive tool for fishery managers. Through the use of categorical variables in a log-linear regression model, we estimate the relationship betwen output (weight of catch) of a single species and input (effort in terms of number of days fished) for a cross section of individual vessels having different capital stocks, temporal and spatial features, national origins, and directed species. Production parameters associated with the different vessel characteristics and the basic relationship between catch and days fished are thus estimated simultaneously. The single input production function which we estimate is given by

$$(1) \quad Q = \alpha\, \mathrm{DF}^{B}\, u$$

or

$$(2) \quad \log (Q) = \log (\alpha) + B \log (\mathrm{DF}) + \log (u)$$

with

$$(3) \quad \log (\alpha) = \alpha_1 + \sum_{i=2}^{k} \alpha_i \mathbf{X}_i$$

where $Q$ is the catch in weight of a given species, DF is days spent fishing by the vessel doing the harvesting, $u$ is a random disturbance, and $\mathbf{X}_i$ is a vector of vessel characteristics including technical, temporal, and spatial factors.

Substituting (3) into (2) we write:

$$(4) \quad \log (Q) = \alpha_1 + \sum_{i=2}^{k} \alpha_i \mathbf{X}_i + B \log (\mathrm{DF}) + \log (u)$$

where $\alpha_1$ is the intercept for a vessel with arbitrary characteristics (i.e. the base vessel) and $\Sigma\alpha_i\mathbf{X}_i$ reflects adjustments made when vessel characteristics differ from the base vessel. The curvature parameter ($B$) represents returns to days fished, and if $B$ is found to be significantly different from one, changing days fished by a given proportion changes output by a different proportion. The curvature parameter ($B$) may in fact be a function of vessel characteristics. By restricting $B$ to be constant we are not allowing for varying returns to days fished and are thus unable to test statistically for varying returns. In general, it would be desirable to interact log (DF) with vessel characteristics ($\mathbf{X}_i$). Given the large $k$ for our empirical analyses specifying $B_i = f(\mathbf{X}_i)$ greatly increases the number of parameters to be estimated and poses severe multicollinearity problems. Alternatively a more general homothetic production function may be tested by estimating $Q\, e^{\theta Q} = \alpha \mathrm{DF}^{B}$ where the curvature parameter now becomes $B/(1 + \theta Q)$ and can vary with output. This alternative, however, causes nonlinearities thus making statistical estimation much more difficult.

Included in the $\mathbf{X}$ vector are variables representing years, months, geographical area, target species, country of vessel registry, and gear and tonnage class of vessels. The $\mathbf{X}$ vector is made up entirely of dummy variables taking on values of 0 or 1. These variables serve to classify the observation according to the above characteristics and allow the parameter ($\alpha$) to change accordingly. The method is commonly referred to in the statistical literature as covariance analysis.

The data were collected by the International Commission for the Northwest Atlantic Fisheries (ICNAF), and provided to us by the National Marine Fisheries Service at Woods Hole, Massachusetts. The ICNAF data record monthly catch of various species by vessels for the years 1960–74, and in addition classify observations by area where caught and numerous characteristics of the vessels. We focus primarily on two subareas of ICNAF area 5 (the Georges Bank and Gulf of Maine) since this area off Cape Cod, Massachusetts, has a long continuum of data, was heavily fished by domestic and foreign fleets, and has been especially important to U.S. and Canadian fishermen since the 19th century. Stocks of cod, haddock, flounder, redfish, and herring have existed with a minimum amount of influence from other areas due to the deep waters that border the area.

In Table 2 one can see the vessel information included in the $\mathbf{X}$ vector as well as the number of vessels for each category. Two ICNAF areas, denoted as 5Z and 5Y, were used by the fleets of vessels in the sample. Since vessels are either in one group or the other, one area must be omitted from the regression to avoid perfect multicollinearity. Area 5Y was arbitrarily chosen for omission from the regression and thus becomes a characteristic of the base vessel whose intercept is given by the constant term of the regression.

*Target species* represent the main species sought by the individual vessel. A unit of effort directed at a given species should result in greater catch of that species than another species. In addition, vessels with different target species may have different by-catch production parameters. To allow for these possibilities, a vector of dummy variables was created which includes all the possible target species (cod, haddock, flounder, redfish, herring, groundfish, and mixed) in our data set. For each vessel observation a value of 1 was assigned to the target species variable corresponding to that designated by the vessel captain and indicated in the ICNAF data. The value 0 was assigned for all other target species variables. In the regression estimations for a given species catch (e.g. cod), that species (e.g. cod) was eliminated from the target species vector. The base vessel for target species is, therefore, a vessel for which the catch of a given species is a directed catch. In a regression for the catch of a given species, vessels for which the catch is directed are included in the observations as well as vessels for which the catch is a by-catch. We are able to distinguish the production parameter for directed versus by-catch by letting any one (or none) of the target species dummy variables be one. If all the target species dummy variables are zero, then the production coefficient represents

TABLE 2. Number of observations in each category.

| Variable | Species | | | | |
|---|---|---|---|---|---|
| | Cod | Haddock | Flounder | Redfish | Herring |
| Area | | | | | |
| 5Y | 677 | 71 | 866 | 46 | 487 |
| 5Z | 4718 | 3712 | 3587 | 1820 | 1229 |
| Target species | | | | | |
| Cod | 842 | 537 | 483 | 119 | 5 |
| Haddock | 601 | 632 | 513 | 327 | 2 |
| Flounder | 979 | 545 | 1188 | 25 | 45 |
| Redfish | 351 | 293 | 264 | 418 | 1 |
| Herring | 70 | 32 | 36 | 24 | 477 |
| Groundfish | 564 | 459 | 441 | 214 | 46 |
| Mixed | 1988 | 1285 | 1548 | 739 | 1140 |
| Vessel tons | | | | | |
| 0–49 | 1905 | 1034 | 1425 | 250 | 409 |
| 50–149 | 1542 | 1138 | 1533 | 648 | 274 |
| 150–499 | 1387 | 1282 | 1118 | 739 | 162 |
| 500–999 | 330 | 217 | 222 | 98 | 295 |
| 1000–1999 | 32 | 10 | 7 | 12 | 181 |
| ≥2000 | 199 | 102 | 168 | 119 | 395 |
| Gear | | | | | |
| Side trawl | 3294 | 2452 | 3113 | 1587 | 556 |
| Midw trawl | 32 | 4 | 5 | 19 | 187 |
| Stern trawl | 960 | 643 | 987 | 253 | 451 |
| Pair trawl | 120 | 82 | 2 | 0 | 300 |
| Dan seine | 15 | 2 | 37 | 0 | 0 |
| Pur seine | 3 | 0 | 4 | 2 | 136 |
| DR gill net | 23 | 13 | 4 | 0 | 10 |
| SN gill net | 160 | 98 | 68 | 4 | 0 |
| Fixed net | 26 | 1 | 28 | 0 | 75 |
| Hand line | 290 | 81 | 42 | 0 | 0 |
| Long line | 320 | 272 | 124 | 0 | 0 |
| Other line | 149 | 134 | 58 | 0 | 0 |
| Misc. | 3 | 0 | 1 | 0 | 1 |
| Country | | | | | |
| U.S. | 4397 | 2973 | 3779 | 1488 | 936 |
| Canada | 681 | 648 | 422 | 226 | 0 |
| USSR | 215 | 122 | 229 | 105 | 371 |
| UK | 5 | 5 | 1 | 0 | 0 |
| France | 4 | 2 | 1 | 0 | 9 |
| West Ger. | 13 | 3 | 1 | 6 | 129 |
| Italy | 63 | 20 | 9 | 28 | 218 |
| Romania | 7 | 9 | 15 | 5 | 28 |
| Japan | 5 | 0 | 9 | 4 | 9 |
| Bulgaria | 5 | 1 | 7 | 4 | 16 |
| Year | | | | | |
| 60 | 140 | 129 | 90 | 61 | 0 |
| 61 | 84 | 83 | 70 | 69 | 25 |
| 62 | 51 | 46 | 32 | 26 | 19 |
| 63 | 151 | 138 | 108 | 74 | 48 |
| 64 | 258 | 242 | 194 | 135 | 34 |
| 65 | 248 | 231 | 185 | 130 | 51 |
| 66 | 311 | 297 | 228 | 124 | 80 |
| 67 | 367 | 336 | 269 | 148 | 70 |
| 68 | 406 | 327 | 322 | 129 | 109 |
| 69 | 405 | 306 | 316 | 120 | 158 |
| 70 | 403 | 248 | 306 | 128 | 233 |
| 71 | 444 | 293 | 402 | 170 | 207 |

Table 2. (*Concluded*)

| Variable | Species | | | | |
|---|---|---|---|---|---|
| | Cod | Haddock | Flounder | Redfish | Herring |
| 72 | 631 | 344 | 552 | 176 | 225 |
| 73 | 723 | 374 | 656 | 189 | 220 |
| 74 | 773 | 389 | 743 | 187 | 237 |
| Month | | | | | |
| Jan. | 412 | 260 | 340 | 126 | 95 |
| Feb. | 421 | 267 | 328 | 136 | 84 |
| March | 422 | 279 | 355 | 134 | 114 |
| April | 448 | 293 | 384 | 140 | 161 |
| May | 477 | 323 | 406 | 143 | 142 |
| June | 464 | 333 | 399 | 137 | 124 |
| July | 438 | 356 | 380 | 150 | 160 |
| Aug. | 445 | 363 | 388 | 178 | 181 |
| Sept. | 443 | 364 | 377 | 185 | 174 |
| Oct. | 478 | 362 | 379 | 199 | 167 |
| Nov. | 491 | 308 | 377 | 177 | 151 |
| Dec. | 456 | 275 | 360 | 161 | 163 |

that of directed catch. If one of the target species variables is one, then the production coefficient represents a by-catch of a given species (e.g. cod) by vessels directing effort at another given species (e.g. flounder). This procedure with a minimal number of parameters allows us to differentiate the effort parameter for target catch from that of by-catch as well as to distinguish among different by-catch effort parameters.

Since our approach ignores the amount and composition of total catch, some information is lost in the estimation procedure. We feel that (1) focusing on the catch of individual species (our dependent variable), and (2) distinguishing between directed catch and by-catch are sufficiently important, however, to fishery managers to support our model. Mundlak (1964), one of the first to discuss the multiple-output problem, proposed a transcendental function which formed a generalization to the Cobb-Douglas production function and allowed levels of all outputs ($Q_i$) to be included in a single production function. His function $Q_1^{\alpha_1} \cdots Q_m^{\alpha_m} e^{\sum B_i \bar{Q}_i} = \alpha DF^{\beta}$ can be linearized in logs and consistently estimated in principle by a simultaneous use of instrumental variables and covariance analyses. Appropriate exogenous instrumental variables like input and output prices, however, are not available in our data set precluding this quite general approach.

*Vessel tonnes* represent the tonnage of the individual vessel with the smallest size (0−49) being the base vessel and thus omitted. The base vessels for *gear* and *country* are side trawlers and the U.S., respectively.

The *year* variables (1960 the base year) are included in the regression to allow for annual differences in catchability. In most economic analyses temporal changes in production parameters indicate technical change. For fisheries, however, the shifts in catchability from year to year probably have more to do with changing fish stock levels. Similarly the *month* variables allow the production parameter to reflect any seasonal effects. January was chosen as the base month.

Ideally one would like independent stock measures derived through biological field studies. Given the enormous cost of this approach for ocean fisheries, the stock externality problem has been traditionally handled by relating current industry catch per unit of effort to a weighted average of past industry effort. (See Gulland (1969), Royce (1972), and Ricker (1975) for more detailed descriptions of this approach.) In a preliminary phase of our work we included a direct measure of stock in the regressions. We used relative abundance indices (measured as stratified mean catch in kilograms per tonne) from the autumn bottom trawl survey conducted by the National Marine Fisheries Service (see Clark and Brown (1977) for details of the sampling procedure). Unfortunately this variable proved insignificant probably because of measurement error as well as lack of variability across observations since the trawl surveys are conducted only once a year and our vessel data are monthly.

## Empirical Findings

The results of estimating equation (4) using ordinary least squares regression for the five species considered are found in Table 3. The dependent variable in each regression is the catch of a given species and the estimated equation is interpreted as a directed catch or by-catch relationship depending on the species designated as the target species. In general the statistical fit is quite good considering that most of the variation in the data is cross-sectional. The $R^2$ (adjusted) ranges from 0.57 to 0.81 for the five species. Where a priori expectations exist for parameter signs, the findings generally confirm these expectations. The constant term represents the power to which the natural logarithm base must be raised to obtain the production coefficient ($\alpha$) for the base vessel. For all species, the base vessel represents the directed catch of a small (0−49 t) U.S. side trawler fishing in January, 1960, in the Gulf of Main (area 5Y).

The variable Log DF (log days fished) for all species is positive and highly significant with $P \leq 0.01$ using a $t$-test. The estimates of $\hat{B}$ range from a low of 0.41 for redfish to a

TABLE 3. Regression coefficients and summary statistics. (*, **Indicate significance at $P \leq 0.05$ and $P \leq 0.01$ levels, respectively, using a two-tailed $t$-test.)

| Variable | Species | | | | |
|---|---|---|---|---|---|
| | Cod | Haddock | Flounder | Redfish | Herring |
| $R^2$ (adjusted) | 0.71 | 0.75 | 0.81 | 0.57 | 0.67 |
| $F$ | 223 | 200 | 325 | 51 | 68 |
| $N$ | 5395 | 3783 | 4473 | 1866 | 1716 |
| Constant | 0.85* | −0.47* | 1.13** | 2.65** | 0.06 |
| Log DF | 0.81** | 0.84** | 0.91** | 0.41** | 0.72** |
| Area | | | | | |
| 5Y | Base | Base | Base | Base | Base |
| 5Z | 0.43** | 1.20** | −0.43** | 0.48* | 0.23* |
| Target species | | | | | |
| Cod | Base | −0.81** | −1.08** | −3.20** | −2.20** |
| Haddock | −0.91** | Base | −1.58** | −3.67** | −4.67** |
| Flounder | −1.62** | −1.69** | Base | −4.36** | −4.14** |
| Redfish | −2.03** | −1.83** | −2.35** | Base | −3.78* |
| Herring | −1.92** | −1.37** | −2.97** | −2.80** | Base |
| Groundfish | −0.80** | −0.72** | −1.21** | −2.55* | −3.39** |
| Mixed | −1.45** | −1.02** | −0.83** | −2.06** | −2.24** |
| Vessel tons | | | | | |
| 0−49 | Base | Base | Base | Base | Base |
| 50−149 | 0.44** | 1.15** | −0.04 | 1.43** | 0.22 |
| 150−499 | 0.58** | 1.66** | −0.45** | 1.30** | 1.74** |
| 500−999 | 0.32** | 1.86** | −0.77** | 2.06** | 2.10** |
| 1000−1999 | 0.66** | 3.31** | 0.28 | 1.31** | 2.36** |
| ≥2000 | 0.12 | 1.50** | 0.28* | 1.42** | 2.80** |
| Gear | | | | | |
| Side trawl | Base | Base | Base | Base | Base |
| Midw trawl | −0.03 | 0.27 | 1.08* | 0.18 | 0.56** |
| Stern trawl | 0.33** | 0.20 | 0.24** | −0.48** | 0.24 |
| Pair trawl | 1.42** | 0.64** | 0.53 | — | 2.31** |
| Dan seine | 0.01 | 1.47* | −0.10 | — | — |
| Pur seine | 0.54 | — | 1.20** | 1.54 | 2.11** |
| DR gill net | −0.06 | −1.30** | −3.76** | — | 0.67 |
| SN gill net | 0.01 | −1.46** | −3.58** | −1.37* | — |
| Fixed net | −2.28** | −1.44 | −3.72** | — | 0.64** |
| Hand line | −0.48** | −1.99** | −2.58** | — | — |
| Long line | 0.09 | −0.14 | −3.02** | — | — |
| Other line | 0.42** | 0.36** | −3.59** | — | — |
| Misc. | 0.40 | — | −0.23 | — | 5.99** |
| Country | | | | | |
| U.S. | Base | Base | Base | Base | Base |
| Canada | −0.17** | −0.09 | −0.93** | −1.08** | — |
| USSR | −1.08** | −1.82** | −1.59** | 0.22 | −0.40** |
| UK | −0.61 | 0.34 | −0.52 | — | — |
| France | −0.05 | 1.08 | −0.70 | — | −0.39 |
| West Ger. | −2.18** | −2.61** | −3.88** | −1.97** | −0.63** |
| Italy | −1.17** | −1.49** | −2.79** | −1.01** | −0.46** |
| Romania | −0.11 | 1.07* | 1.04** | −0.29 | −1.95** |
| Japan | −0.44 | — | −3.12** | −1.38* | −1.52** |
| Bulgaria | −2.05** | −2.01 | −2.13** | −0.94 | −0.39 |
| Year | | | | | |
| 60 | Base | Base | Base | Base | Base |
| 61 | −0.06 | −0.13 | 0.48** | 0.21 | — |
| 62 | 0.45** | 0.25 | 0.80** | 0.22 | −2.13** |
| 63 | 0.35** | 0.18 | 0.31* | −0.21 | 1.18** |
| 64 | −0.01 | 0.12 | 0.50** | −1.09** | 1.71** |

TABLE 3. (*Concluded*)

| Variable | Species | | | | |
|---|---|---|---|---|---|
| | Cod | Haddock | Flounder | Redfish | Herring |
| 65 | 0.07 | 0.06 | 0.51** | −1.30** | 0.45 |
| 66 | 0.16 | 0.05 | 0.45** | −0.97 | 0.95** |
| 67 | 0.05 | −0.49** | 0.63** | −0.93** | 1.00** |
| 68 | 0.24* | −0.43** | 0.51** | −0.99** | 1.32** |
| 69 | 0.26** | −0.77** | 1.00** | −0.73** | 2.16** |
| 70 | 0.21* | −1.08** | 0.77** | −0.32 | 2.14** |
| 71 | −0.03 | −1.34** | 0.62** | −0.60** | 1.87** |
| 72 | −0.28** | −1.55* | 0.43** | −1.19** | 1.47** |
| 73 | −0.19* | −1.64** | 0.30** | −0.96** | 1.18** |
| 74 | −0.24* | −1.76** | 0.21 | −1.08** | 1.24** |
| Month | | | | | |
| Jan. | Base | Base | Base | Base | Base |
| Feb. | −0.02 | 0.06 | −0.10 | 0.21 | 0.26 |
| March | 0.00 | 0.05 | 0.00 | 0.54** | 0.21 |
| April | −0.04 | 0.15 | 0.06 | 0.21 | −0.23 |
| May | −0.15* | 0.08 | 0.09 | 0.47** | −0.27 |
| June | −0.10 | −0.05 | 0.04 | 0.23 | −0.32 |
| July | −0.18** | −0.09 | −0.12 | −0.13 | 0.08 |
| Aug. | −0.25** | 0.03 | −0.21** | 0.05 | 0.29 |
| Sept. | −0.23** | −0.03 | −0.09 | 0.11 | 0.61** |
| Oct. | −0.12 | 0.01 | −0.13 | −0.05 | 0.44* |
| Nov. | −0.18** | −0.19* | −0.04 | −0.22 | −0.37 |
| Dec. | −0.11 | −0.27** | −0.00 | −0.10 | −0.18 |

high of 0.91 for flounder which, given the low standard errors of $\hat{B}$, indicates a significant departure from linearity for all species (i.e. decreasing returns to days fished). Thus twice as many days fished with the same vessel and crew yields less than twice the catch. This result indicates the presence of some limiting factor in the production process. One possibility is the existence of decreasing returns to scale since days fished and gross tonnage are usually positively correlated. Another interpretation involves stock and congestion externalities which are difficult to measure directly. Fixed fish stocks and surface area limit the ability to increase catch by simply increasing days fished. Thus a curvature parameter ($\hat{B} < 1$) may be more of an indication of diminishing returns to a variable factor (days fished) given the fixed factors, such as fish stock and surface area. Evidence for this conclusion was found in our preliminary estimations when relative abundance indices were included as a variable in the log-linear model. Although insignificant the presence of the stock variable increased the curvature parameter ($\hat{B}$) to above one. Since stock cannot be controlled in the short run, we feel that diminishing returns to days fished ($\hat{B} < 1$) is probably more realistic.

Returns to gross tonnage are reflected by the coefficients of the tonnage class variables. These coefficients are generally positive and highly significant with the exception of the intermediate vessel size-classes used in the harvest of flounder. For herring and haddock vessels up to 1999 t, the coefficient increases for larger vessels (i.e. higher tonnage class) indicating an increased production parameter ($\log \alpha$) for larger vessels. For cod and redfish, vessels larger than 49 t exhibit greater catchability than the base vessel (0−49 t).

The area variable is significant for all species. For cod, haddock, redfish, and herring, area 5Z (the Georges Bank) is more productive whereas for flounder, the base area (area 5Y or Gulf of Maine) is more productive. The gear, country, year, and month variables have mixed results. Given the classifications present in the ICNAF data, these variables are included to allow for the possibility of different effort parameters with no theoretical a priori expectations on parameter signs. *F* tests were performed on the groups of classificatory of dummy variables indicating that in some cases these classifications could be ignored, and the intercept could be pooled within a group of heterogeneous vessels. However, since there is no consistency across species about which groups are needed, we choose to retain all groups for all species.

The gear variables indicate that variation in productivity across gear, holding other characteristics constant, is sometimes quite pronounced. Likewise the country variables indicate significant variations in catchability across countries with U.S. vessels generally most productive holding other variables constant. The month variables are generally not significant although for all the species there is at least 1 month for which seasonality is important.

The year variables are included to allow the production function to shift over time reflecting changes in technology, fishermen's learning, and fish stock. Any trend or regularity in the year parameters can be very useful for predicting catchability and thus managing the resource. Although not available for our data, information on the age of the vessel and gear, vessel horsepower, and learning would be helpful in reflecting technological change and thus allow the year variables to reflect only stock changes. Even though they reflect a mixture of factors, the year variables are in general quite significant. For cod, haddock, and redfish, catchability is

falling over time with haddock and redfish having the most pronounced negative temporal effect. The severe depletion of the stock of haddock led to quotas being first imposed in 1970 and lowered further in 1972 both contributing to a lower effort parameter in the later years. For flounder and herring, catchability increased from 1960 to 1974 although in both cases the temporal effect has weakened since 1969. Quotas on yellowtail flounder (*Limanda ferruginea*) were in fact introduced on the Georges Bank in 1971, which may explain the decline in the year variable in the early 1970's.

The target species variables are all negative and significant at $P \leq 0.05$ level using the $t$-test. The production parameter is thus always lower for by-catch than for target or directed catch. Except for days spent fishing, the target species of the captain is the most important variable in determining catch. The consistency of the target species variables suggests that vessel captains are accurate in revealing their intentions and also able to catch what they seek.

This assumes that target species is in fact determined ex ante (i.e. before catch takes place), most likely a function of relative profitability among the species. Carlson (1973) explicitly raises the issue of fishermen behavior in comparing the New England trawl fishery with the Gulf tuna seine fleet. He argues that in the tuna fishery a joint product model applies where vessel captains have limited control over what species they catch because of considerable mixing of species, and although the location of fish may be generally known, the species are joint products. The mix actually caught is an act of nature and beyond the captains' control. In the trawl fishery, however, skippers exert considerable control over the species caught by directing effort towards those species providing the highest expected profit and have considerable ability to control the species mix while on the fishing ground. Our findings lend further support for Carlson's hypothesis concerning the New England trawl fishery.

## Applications for Management

Since the work by Gordon (1954) on the economics of common property fisheries, it is generally accepted that government intervention is a necessary, although not necessarily a sufficient, condition of efficient utilization of fisheries. Gordon and others who have followed (Anderson 1977 and Clark 1976) have demonstrated that with no regulation too many resources will be allocated to the utilization of a fish stock. That is, a reduction in effort applied to the fish stock will allow for a net increase in the value of goods and services produced in the economy. This is possible because an open-access fishery will operate where the value of the marginal product of effort is less than the opportunity cost of effort.

Practical application of the results of this analysis in an actual fisheries management program will enable management authorities to calculate the value of the marginal product of fishing effort so as to determine the optimal effort for a given fishery. The U.S. Fisheries Conservation and Management Act of 1976 established Regional Management Councils and required them to develop fishery management plans for each important commercial and recreational fishery in their area. As might be expected, the charge to the councils was not to achieve economic efficiency in the operation of the fishery, but rather to achieve "optimal yield." "Optimal yield" is defined as maximum sustainable yield as modified by social, economic, and ecological factors.

Our empirical results will be of use to these management councils in fulfilling their legislative obligations. The following are some of the specific uses:

1) They will be an aid in determining the short-run maximum economic yield of the fishery.

2) They will be an aid in considering certain other social factors and thus can provide information useful in determining "optimal yield."

3) They can be used as an aid in showing the effects of the quarterly quotas which are currently being used by the New England Council to manage cod, haddock, and yellowtail flounder.

4) They will be useful when analyzing different types of limited entry programs.

Each of these uses will be described below. The analysis will be necessarily brief because of space limitation.

### Determine Maximum Economic Yield

Assuming there is only one independent species under consideration, a vessel production function of the following form can be generated from the above results.

$$Q = e^{(\alpha + \Sigma a_i \mathbf{X}_i)} \, DF^{B}$$

where $\mathbf{X}_i$ is the vector of vessel characteristics and time and spatial variables.

If the management council has a comparison of the current stock and environmental conditions with that of previous years (e.g. the concensus of the experts may be that the stock situation is quite similar to that which existed in 1969), then by making the appropriate modification in the coefficient of the year variable, the function can be adjusted for the state of the fish stock. Although we have indicated that absolute estimates of stock are in fact difficult to obtain, relative or comparative estimates seem within the biological state of the art. If there are cases where they are not, then a second best alternative is to use the coefficient of the most recent year.

Keeping the example very simple, assume that effort can be produced by only three fishing vessels of different types. The assumption of only one boat in each class is obviously overly simplistic, but it does allow for a clear presentation of the principle involved. For practical application it would be necessary to take into account such things as (1) constraints on the days fished by each vessel type due to weather, repair, etc., (2) the necessity of allowing the number in each vessel type to be a variable, (3) the direct catch vs. by-catch problem, and (4) more direct consideration of other externalities.

Each vessel can be described by its characteristics and the month in which it fishes (i.e. that part of the **X** vector other than year fished). It will then be possible to obtain a production function for each of these types of effort. Their summation will be a yield curve for the fishery as a whole. By estimating cost functions for each of these vessel types, a profit function for the fishery of the following form may be

obtained:

$$\text{Profit} = P \sum_{j=1}^{3} Q_j - \sum_{j=1}^{3} C_j(\text{DF}_j)$$

where the $C_j$ are the cost functions for the three vessels, and $P$ is unit price of fish. It must be remembered, it is the profit function of the fishery as a whole, not a profit function for an individual vessel. Using nonlinear procedures it will be possible to solve for the combination of days fished of each type of effort that will maximize profits. If the only goal of the management council is to obtain short-run economic efficiency, then the solution to the above maximization problem will tell them what combination of effort to use in the current year. By definition, the short-run production functions will not be able to trace the relationship between effort in 1 year, and stock size and hence catch in another. Admittedly an analysis of maximum economic yield which considered these long-term effects would be superior. However, the Councils are likely to work on a year-by-year basis in any event, so the short-run analysis will not be without merit.

### Provide Information on "Optimal Yield"

If the Councils have goals other than strict economic efficiency, however, other combinations of effort which are of interest to the Council may be substituted into the profit function and the change in profit, in physical yield of catch, and in the distribution of the gains from the fishery can be noted. For example, the economically efficient combination may not include small inshore vessels, but the Council may, for equity reasons, desire that a portion of the catch be allocated to them. The above analysis will allow the Councils to see the trade-off between economic efficiency and the distribution of income among the fleet or other management goals. This will be of some assistance to them in determining optimal yield. The above methodology can be generalized to the case of interdependent fisheries with catch and by-catch equations. The manipulations will be somewhat more difficult but the procedure will be essentially the same.

### Show Effects of Current Quarterly Quota Programs

As part of the current management program of the New England council for cod (as well as other groundfish), quarterly quotas have been set and broken down according to vessel size, season, and area. See Table 4 for 1980–81 quotas. In general we would oppose such a system because it does not address the real problem of fleet over capitalization, but empirical results nevertheless can be used to show the effects of this policy. We use the cod harvest equation to determine the days fishing required for the U.S. fleet directing effort towards cod to fulfill the quotas. Similar calculations could be performed for other species. We proceed as follows beginning from the cod base vessel equation found in Table 3.

$$\log(Q) = 0.85 + 0.81 \log(\text{DF}).$$

Assuming a U.S. stern trawler and that 1974 is the best estimate for trend levels, we then adjust the logarithm of the base production coefficient (0.85) by the factors 0.33 and −0.24. The symbol α becomes 0.94 (0.85 + 0.33 − 0.24). Since quota limitations are imposed for calendar quarters, for the month variable we take the 3-mo average of the coefficients of the monthly variables to calculate an appropriate effort parameter for a quarter. These adjustments to the base vessel equation given above become −0.01, −0.10, −0.22, and −0.14 for the first, second, third, and fourth quarters, respectively. Table 5 presents the results of the adjustments made above to log α for the areas and vessel size-classes in our regression model closely corresponding to those for which there are specific quotas. We illustrate the calculation of log α for a U.S. (50–149 t) stern trawler fishing in the Georges Bank (area 5Z) during the third quarter of 1974. The base intercept from Table 3 (0.85) becomes 1.59 (i.e. 0.85 + 0.33 − 0.24 − 0.22 + 0.43 + 0.44). Thus

TABLE 4. Quarterly quotas in tons for cod using mobile gear.[a]

| Quarter and area | Vessel size-class (gross tonnes) | | |
|---|---|---|---|
| | 0–60 | 61–125 | Over 125 |
| Fourth quarter 1980 | | | |
| Gulf of Maine | 921 | 542 | 541 |
| Georges Bank | 675 | 2393 | 3983 |
| First quarter 1981 | | | |
| Gulf of Maine | 1106 | 438 | 270 |
| Georges Bank | 798 | 2109 | 2865 |
| Second quarter 1981 | | | |
| Gulf of Maine | 1265 | 415 | 88 |
| Georges Bank | 873 | 3006 | 3266 |
| Third quarter 1981 | | | |
| Gulf of Maine | 757 | 420 | 88 |
| Georges Bank | 491 | 1832 | 3184 |

[a]Source: *Federal Register*, Vol. 45, No. 192, Oct. 1, 1980, p. 64998, Appendix A.

TABLE 5. Production parameters (ln α) for U.S. stern trawlers catching cod given selected quarters, areas, and vessel types.

| Quarter and area | Vessel size-class (gross tonnes) | | |
|---|---|---|---|
| | 0–49 | 50–149 | 150–499 |
| First quarter | | | |
| 5Y[a] | 0.93 | 1.37 | 1.51 |
| 5Z[a] | 1.36 | 1.80 | 1.94 |
| Second quarter | | | |
| 5Y | 0.84 | 1.28 | 1.42 |
| 5Z | 1.27 | 1.71 | 1.85 |
| Third quarter | | | |
| 5Y | 0.72 | 1.16 | 1.30 |
| 5Z | 1.15 | 1.59 | 1.73 |
| Fourth quarter | | | |
| 5Y | 0.80 | 1.24 | 1.38 |
| 5Z | 1.23 | 1.67 | 1.81 |

[a]5Y and 5Z represent the Gulf of Maine and Georges Bank, respectively.

the estimating production equation for this category of vessels becomes

(5) $\log (Q) = 1.59 + 0.81 \log (\mathrm{DF})$.

To predict catch ($Q$) we need to take the antilog of log ($Q$). When this is done a positive bias is introduced since

$$E(\text{antilog log } Q) = [\text{antilog } E(\log Q)]\left(1 + \frac{\sigma^2}{2}\right)$$

where $E$ is the expectations operator and log $Q$ is a random variable distributed lognormally with variance $\sigma^2$ (see Goldberger 1964). To remove the bias in predicting $Q$ one must use as an estimate for $Q$ the following equation

$$Q = (\text{antilog log } \alpha)\,\mathrm{DF}^B \Big/ \left(1 + \frac{\sigma^2}{2}\right).$$

We estimate $\sigma^2$ as the variance of the fitted value of log $Q$ over the data employed to estimate the models in Table 3. The variance of log $Q$ was estimated as 2.20, 3.13, 3.35, 2.15, and 4.28 for cod, haddock, flounder, redfish, and herring, respectively.

Continuing our illustration for cod and the vessel described by Eq. (5), we estimate the adjusted production parameter ($\alpha$) as

$$(\text{antilog log } \alpha)\Big/\left(1 + \frac{\sigma^2}{2}\right) = (\text{antilog } 1.59)\left(1 + \frac{2.20}{2}\right)$$

Thus the equation appropriate for predicting the catch of a U.S. (50–149 t) stern trawler fishing area 5Z in the third quarter of 1974 is

$$Q = 2.34\ \mathrm{DF}^{0.81}.$$

Table 6 presents the results of the calculation of $\alpha$ for vessel sizes, areas, and seasons included in Table 5. The entries in Table 6 may be viewed as statistically consistent estimates of the production parameter ($\alpha$) for various vessel categories.

Given the production parameters in Table 6, the quarterly quotas in Table 4, and assumptions about the fleet size ($V$), one can calculate the fishing days necessary for the fleet to land the quota. The classifications in Table 4 come as close as possible to the groupings for effort parameters found in Table 6. Stern trawlers represent a mobile gear, ICNAF areas 5Y and 5Z closely match the Gulf of Marine and the Georges Bank, and the gross tonnages are quite similar since few U.S. vessels exceed 499 t.

To find the catch for a fleet of vessels it is only necessary to multiply the catch of a single vessel given by Eq. (1) by the number of vessels in fleet ($V$). Setting the fleet catch equal to the fleet quota ($Q^*$) and assuming $E(u) = 0$, we obtain

(6) $Q^* = V\alpha\mathrm{DF}^B$.

Assuming equal quotas per vessel within a class, we obtain the quota per vessel

(7) $Q^*/V = \alpha\mathrm{DF}^B$.

TABLE 6. Adjusted production parameters ($\alpha$) for U.S. stern trawlers catching cod given selected quarters, areas, and vessel types.

| Quarter and area | Vessel size-class (gross tonnes) 0–49 | 50–149 | 150–499 |
|---|---|---|---|
| First quarter | | | |
| 5Y[a] | 1.21[b] | 1.87 | 2.16 |
| 5Z[a] | 1.86 | 2.88 | 3.31 |
| Second quarter | | | |
| 5Y | 1.10 | 1.71 | 1.97 |
| 5Z | 1.70 | 2.63 | 3.03 |
| Third quarter | | | |
| 5Y | 0.98 | 1.52 | 1.75 |
| 5Z | 1.50 | 2.34 | 2.69 |
| Fourth quarter | | | |
| 5Y | 1.06 | 1.65 | 1.89 |
| 5Z | 1.63 | 2.53 | 2.91 |

[a]5Y and 5Z represent the Gulf of Maine and Georges Bank, respectively.

[b]We compute $\alpha$ by dividing antilog (log $\alpha$) from Table 5 by $[1 + (2.20/2)] = 2.10$.

TABLE 7. Cod fleet effort needed to fulfill quarterly quotas. (Days fished for each vessel, in a fleet of 30 vessels for each size-class.)

| Quarter and area | Vessel size-class (gross tonnes) 0–60 | 61–125 | Over 125 |
|---|---|---|---|
| Fourth quarter 1980 | | | |
| Gulf of Maine | 44.7 | 13.5 | 5.1 |
| Georges Bank | 17.9 | 49.7 | 36.9 |
| First quarter 1981 | | | |
| Gulf of Maine | 47.6 | 8.9 | 4.1 |
| Georges Bank | 18.7 | 36.2 | 44.5 |
| Second quarter 1981 | | | |
| Gulf of Maine | 63.2 | 9.3 | 1.1 |
| Georges Bank | 23.4 | 62.7 | 58.4 |
| Third quarter 1981 | | | |
| Gulf of Maine | 38.7 | 10.9 | 1.3 |
| Georges Bank | 13.4 | 39.3 | 65.5 |

Solving Eq. (7) for DF yields the days necessary for each vessel (or alternatively the fleet) to land the quota. This is done in Table 7 for each of the quotas and vessel classes found in Table 4. We illustrate the calculation for a (0–60 t) vessel fishing on the Georges Bank during the first quarter of 1981. Setting $Q^* = 798$, $V = 40$, and using appropriate parameter values (i.e. $\alpha = 1.86$ and $B = 0.81$), we solve Eq. (7) using logarithms for DF which results in 18.7 DF per vessel needed to fulfill the quota for this classification.

Our estimate of a fleet size of 40 for all vessel classes is arbitrary and possibly conservative. This simple illustration, however, indicates that for some vessel categories the quar-

terly quota may be fulfilled in far less time than the fleet may wish to work. This means that there will be a large amount of enforced idle capacity of the fleet. Obviously fishermen will not take kindly to such regulations and there will be intense political pressure for relaxation of the quotas or for new regulatory procedures altogether. Relaxation has already occurred since the quotas in Table 4 represent substantial increases (in some cases >50%) over the earlier years. Fishermen will more than likely react to quotas and their enforced idleness by changing the proportion of their trips directed to a particular target species. If such moves can be predicted, it will be possible to predict how reactions to the quotas will affect the fishery given the production function estimates.

### Analyze Different Types of Limited Entry Programs

In contrast to the quota system discussed above, certain types of regulation procedures restrict entry into the fishery directly. These types of controls are called limited entry programs, and, in general, they are the types most favored by economists. For more detail on limited entry programs, their strengths and weaknesses relative to other regulation devices such as quotas, and problems of implementing them, see Pearse (1979). There are many different types of limited entry programs and the best one to use in any particular fishery depends upon many factors. Our production function estimations will allow for a comparison of certain aspects of at least three different types of limited entry programs.

The first type of limited entry program is taxes on landings. The idea is to reduce the fleet to its optimum size and in the process to force out the least efficient vessels. Vessel production functions, when combined with proper price and cost information, will show how different vessels will react to certain taxes and should provide basic information on which vessels will leave the fishery. As vessels leave the fishery, proper updating of the results will allow for predictions concerning vessel profit levels over the life of the program.

Another type of limited entry program is fisherman's quotas. Under this program, rights to harvest certain amounts of fish are distributed. The theory behind this is that the individual owner of a specific right to harvest will arrange his activities so that he can harvest his allotted amount in a least cost manner. If the rights are transferable, a market will develop in the rights such that they will tend toward those who are the most efficient in the production of effort. It is often argued that fisherman's quotas are inappropriate for fisheries where by-catch is common, and it may well be the case. Nevertheless, use of production equations like these estimated in Table 3 may allow for the development of a system where by-catch can be directly considered in the definition of the fishing rights.

Related to fisherman's quotas is an effort share program in which rights to a specificied amount of fishing time are distributed. Obviously, such a program is not feasible without a means of comparing fishing days of various types of fishing vessels. But this is precisely what production functions do. Thus, knowledge of production parameters facilitates the development of programs that allow for the appropriate amount of fishing effort to be allocated. Also, it will be possible to translate a day's fishing by one type of vessel into a day's fishing by another type so that these effort rights can be made transferable. It must be noted that while our model allows for comparisons, a day's fishing by a certain vessel type at a particular month and at a particular location, the space parameter may be too large to be of much use. If data on fishing location could be obtained on a much smaller grid, the usefulness of the results would be enhanced.

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# Twilight Activities and Temporal Structure in a Freshwater Fish Community

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HELFMAN, G. S. 1981. Twilight activities and temporal structure in a freshwater fish community. Can. J. Fish. Aquat. Sci. 38: 1405 – 1420.

Underwater observations of temperate lake fishes suggest that their twilight activities are basically similar to those described by other researchers for coral reef fishes. At dusk, diurnally active lake fishes progressively increased swimming, ceased feeding, disbanded groups, slowed swimming, and finally stopped. At dawn, this sequence was reversed, although at lower light levels than corresponding dusk behaviors. Aggressive, cleaning, and comfort behavior increased at dusk, and fishes changed from group to individual behaviors. By day, nocturnally active fishes rested singly or in aggregations. Predators were apparently more active during twilight. Older individuals of several species were active later in the evening or were increasingly nocturnal in their activities. Characteristic diel and twilight patterns broke down during the breeding season. Temperate lake species differed from coral reef fishes in that they were less attracted to physical structure at night, twilight migrations were less common, and no apparent "interim period" occurred between cessation of activity by diurnal fishes and initiation of activity by nocturnal ones. In general, twilight changeover in the temperate system was more variable and prolonged. These differences may be due to lake fishes having evolved under variable conditions of community diversity, water clarity, predator abundance, and twilight length. Such factors are more constant or predictable in coral reef communities, where twilight activity patterns appear more precise.

*Key words*: changeover, diel, lake, latitude, light, New York, ontogeny, predation, temperate/tropic comparison

HELFMAN, G. S. 1981. Twilight activities and temporal structure in a freshwater fish community. Can. J. Fish. Aquat. Sci. 38: 1405 – 1420.

Des observations sous-marines de poissons de lacs tempérés donnent à penser que leurs activités crépusculaires sont fondamentalement les mêmes que celles de poissons de récifs coralliens décrites par d'autres auteurs. À l'aurore, les poissons lacustres à activité diurne ont une nage graduellement plus rapide, cessent de s'alimenter, se dispersent, ralentissent leur nage et, finalement, s'arrêtent. Au crépuscule, cette séquence est inversée, bien qu'à des niveaux d'éclairage inférieurs à ceux qui accompagnent les comportements crépusculaires correspondants. Au crépuscule, les comportements d'agression, de nettoyage et de confort sont plus prononcés, et les poissons passent de comportements de groupe à des comportements individuels. Le jour, les poissons à activité nocturne se reposent seuls ou en groupes. Les prédateurs sont apparemment plus actifs au crépuscule. Chez plusieurs espèces, les vieux sujets prolongent leur activité plus avant dans la soirée ou deviennent de plus en plus nocturnes. Pendant la saison de ponte, les habitudes nycthémérales et crépusculaires caractéristiques s'estompent. Contrairement aux poissons des récifs coralliens, les espèces des lacs tempérés sont moins attirées la nuit par la structure physique, les migrations crépusculaires sont moins communes et il ne semble pas y avoir de «période intérimaire» entre l'arrêt de l'activité des poissons diurnes et le début de l'activité des poissons nocturnes. En général, la transition crépusculaire dans le système tempéré est plus variable et plus longue. Ces différences peuvent être dues au fait que les poissons lacustres ont évolué dans des conditions variables de diversité de communauté, de clarté d'eau, d'abondance de prédateurs et de durée de crépuscule. Ces facteurs sont plus uniformes ou plus prévisibles chez les communautés des récifs coralliens, où les schémas d'activités crépusculaires semblent plus précis.

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MOST fishes forage primarily during the day, night, or twilight periods. As a consequence, fish communities can generally be characterized by their temporal organization or structure (Emery 1978). Such temporal organization has been described for tropical marine, tropical freshwater, temperate marine, and temperate freshwater assemblages (reviewed by Helfman 1978). Behavioral and physiological events during evening and morning twilight may determine both general diel activity periods and times of onset and cessation of activity of fishes in a community (Hobson 1972; Munz and McFarland 1977). During twilight, diurnally active and nocturnally active species engage in characteristic transitional behaviors as they "changeover" between foraging and resting modes. The distinctiveness of these activities, and the influence of twilight events on temporal structure in a community, may be a response to selection pressures exerted by predators, which are usually most active and successful during twilight periods (Protasov 1966; Woodhead 1966; Hobson 1972).

Studies of changeover phenomena have been largely restricted to the tropical marine fish communities of Hawaii, the Virgin Islands and Cuba, and on the Great Barrier Reef (Hobson 1972; Collette and Talbot 1972; Domm and Domm 1973; Sbikin 1977). Dawn and dusk behavioral events in all three regions were surprisingly similar, despite differences in community composition, reef structure, and techniques of observation.

The twilight activities of otherwise well-studied North American freshwater fishes have remained largely uninvestigated. This study involves underwater observations of diel activity patterns, emphasizing light and time correlates of twilight changeover behaviors in a temperate lake community. I present community-wide behavioral patterns, followed by accounts of individual species. I then compare these with reported changeover phenomena in tropical reef communities, and relate the results to ecological factors, such as predation, that might influence temporal organization in fish communities.

## Study Site and Methods

Most observations were made underwater in <2.5 m water in a 1.3-ha bay in Cazenovia Lake, Madison County, New York (42°56′N, 75°52′W). Cazenovia Lake is a 475-ha, mesotrophic, dimictic lake with a maximum depth of 14 m, although dense macrophytes do not occur below 5 m. The lake is typically ice free from April to December. Water clarity (horizontal Secchi disc visibility) in the littoral zone averaged 4.6 m (SD = 1.2 m, $n$ = 50) during summer months. The fish community of Cazenovia consists of 18 species; twilight activities were recorded for 14 species (Table 1). Additional observations were made in Mirror Lake, Grafton County, New Hampshire (43°56′N, 71°42′W) and Skaneateles Lake, Onondaga County, New York (42°48′N, 76°17′W).

TABLE 1. Fishes of Cazenovia Lake for which twilight data were obtained. Temporal designations refer to primary feeding times of adults. Densities are monthly averages based on 39 daytime and 22 nighttime transect counts, Apr.–Nov. 1976, corrected for search effort (Helfman 1979a). Density value given is the greater of diurnal (D) or nocturnal (N) counts. $n$ = number of months when a species was seen on transects; mean densities were calculated only for those months.

| Scientific name (abbreviation) | Common name | Density (fish/m$^2$) $\bar{x} \pm$ SE ($n$) |
|---|---|---|
| | *Diurnal species* | |
| Esocidae | | |
| *Esox niger* (En) | Chain pickerel | 0.001±0.0002 (7D) |
| Cyprinidae | | |
| *Notropis volucellus* (NV) | Mimic shiner | 0.04±0.02 (5N) |
| *Pimephales notatus* (Pim) | Bluntnose minnow | 0.004±0.004 (2D) |
| Cyprinodontidae | | |
| *Fundulus diaphanus* (Fd) | Banded killifish | 0.002±0.0007 (5D) |
| Centrarchidae | | |
| *Lepomis gibbosus* (Lg) | Pumpkinseed sunfish | 0.06±0.01 (7D) |
| *Lepomis macrochirus* (Lm) | Bluegill sunfish | 0.14±0.04 (7D) |
| *Micropterus dolomieui* (Md) | Smallmouth bass | 0.004±0.002 (4D) |
| Percidae | | |
| *Perca flavescens* (Pf) | Yellow perch | 0.06±0.02 (7D) |
| | *Nocturnal species* | |
| Cyprinidae | | |
| *Notemigonus crysoleucas* (Nc) | Golden shiner | 0.02±0.009 (3D) |
| Ictaluridae | | |
| *Ictalurus nebulosus* (In) | Brown bullhead | 0.001±0.001 (3N) |
| Centrarchidae | | |
| *Ambloplites rupestris* (Ar) | Rock bass | 0.03±0.005 (8N) |
| *Micropterus salmoides* (Ms) | Largemouth bass | 0.01±0.003 (7D) |
| *Pomoxis nigromaculatus* (Pn) | Black crappie | 0.009±0.008 (5D) |
| Percidae | | |
| *Stizostedion v. vitreum* (Sv) | Walleye | 0±0 (0) |

Changeover data were obtained during late spring, summer, and early autumn, while following individual fishes through a twilight period. Many individuals were recognized by coded Tygon tube tags (see White and Beamish 1972). Fish sizes are given as centimetres total length, estimated visually in the field and verified on tagged fishes of known lengths.

Twilight data are drawn from 114 dusk and 42 dawn dives, representing ~300 h of observation. Statements concerning day/night and seasonal fish distribution patterns are based on sightings of >20 000 individuals during 66 daytime and 40 nighttime transect counts along five 4-m-wide transects in the study area (see Helfman 1979a). Transects were superimposed on a grid system of 10 m × 10 m quadrats. Total observation time for this study was ~700 h.

Downwelling luminance was measured with a Gossen Lunapro light meter in an Ikelite underwater housing, calibrated with a Gamma model 3000 SR recording spectroradiometer (see McFarland et al. 1979). Measurements were made at the site of a behavioral observation or 0.5 m below the water surface. Measurements have been converted to approximate lux using conversion factors provided by the manufacturer. "Darkness" (~$2 \times 10^{-3}$ lx) occurred about 45 min after sunset. Light values <$2 \times 10^{-3}$ lx were extrapolated from an evening's light curve and are, therefore, approximate. Additional details of light measurement are given in Helfman (1979a).

Times of behaviors were recorded to the nearest minute. Times of sunrise, sunset, and twilight were calculated from the *American Ephemeris and Nautical Almanac* (Anon. 1974), corrected for longitude and latitude. Times are given with respect to sunset and sunrise; minus (−) values are before and plus (+) values are after sunset and sunrise. Astronomical twilight length varied from 96 to 144 min (mean = 118 min) during the study, complicating comparisons within and between species. Times are, therefore, reported as percentage astronomical twilight, i.e. standardized to 100 min astronomical twilight (e.g. an activity at 30 min after sunset when twilight lasted 118 min = "SS + 25").

## Observations

### Community Events at Twilight

Certain twilight activities characterized most Cazenovia species and are described below. Details of the behavior of individual species follow. Throughout this paper, YOY = young-of-the-year, PYOY = post-young-of-the-year.

*Transition from day to night* — Six activity patterns typified evening changeover (Fig. 1). These patterns are presented in sequence of occurrence. *(1) Increased movement* — Groups of four species (mimic shiner, YOY golden shiner, YOY largemouth bass, and YOY and PYOY yellow perch) and solitary individuals of other species and size-classes increased their swimming speed or distance traversed. This event was determined by (1) the time of maximal swimming speed in followed individuals (18 fish), (2) the time at

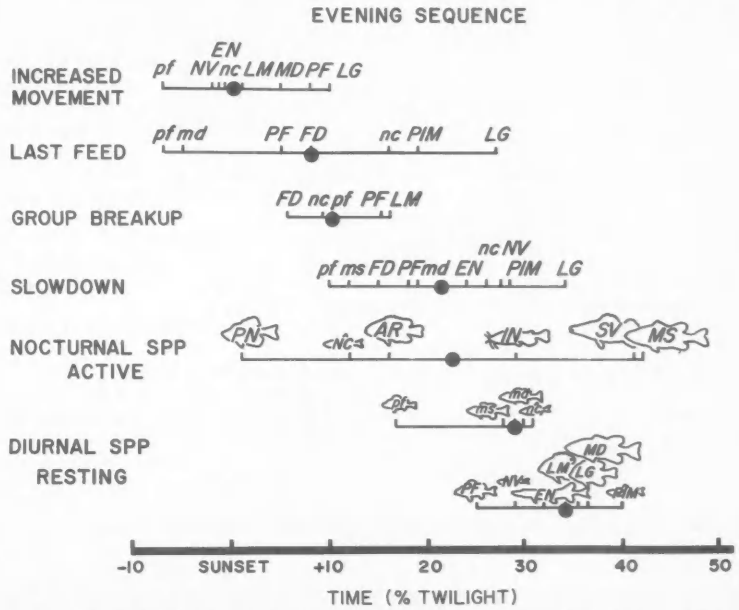


FIG. 1. Sequence of changeover events in the Cazenovia Lake fish community during evening twilight as a function of time relative to sunset. Species are abbreviated as in Table 1; capital letters are for PYOY, lower case for YOY. See Table 2 for details.

which the greatest number of individuals passed a fixed locale (22 occasions), or (3) the approximate time of greatest apparent activity of followed individuals (12 fish).

*2) Group breakup* — Eight Cazenovia species formed diurnally foraging aggregations that disbanded during twilight. This event was determined by the time at which a followed individual departed from a group (23 observations) or the last time a group under observation was sighted (7 observations).

*3) Last feed* — Feeding attempts consisted of bites directed at the substrate or at objects in the water column. The end of foraging was defined by the last time a followed individual attempted to feed (23 occasions).

*4) First stop or slowdown* — Most diurnal fishes did not cease all swimming activities suddenly. Instead, they often (18 observations) went through one to three stops of increasing duration (1–8 min, mean = 3.5 min) prior to a final stop for the evening. On 20 other occasions, followed individuals markedly decreased swimming speed prior to the series of stops or before final stop (= "slowdown").

*5) Initiation of activity in nocturnal fishes* — As diurnally foraging groups disbanded and smaller diurnal fishes began to slow down, individuals from aggregations of nocturnal fishes (golden shiner, rock bass, black crappie) moved to foraging areas. Initiation of activity in rock bass and black crappie was noted as the time at which the last individual left a stationary aggregation. Initiation of activity was different in golden shiner, which formed mobile daytime aggregations, and in brown bullhead and walleye, which rested motionless in benthic vegetation. For these three species, the start of nocturnal activity was recorded when the first active, solitary fish was observed. These times are probably later than the actual times of initiation of activity.

*6) Final stop* — The final event during evening twilight was cessation of activity by diurnal fishes. Most individuals dropped in the water column to within 15 cm above the bottom or above vegetation. The time of this event was determined by (1) when a followed individual ceased movement (36 occasions) or (2) when the first resting individual of a species was encountered (41 occasions). Final stop was verified by observing a resting fish for ≥10 min and by returning to the observation site 15–30 min later (for tagged or distinctively marked individuals).

*Transition from night to day* — Dawn activitives were basically a mirror image of dusk activities and the labels given to activities reflect these similarities (Fig. 2). *(1) First movement* — Fish either initially moved slightly (<5 cm) up into the water column and turned or began swimming very slowly along the bottom.

*2) Speedup* — Speedup was recorded as the initiation of movement along the bottom for fishes already in the water column or when an unmistakable increase in speed occurred for other individuals.

*3) First group* — Group formation was recorded when a followed individual joined conspecifics (6 occasions) or when the first aggregation of a species was encountered (17 occasions).

*4) First feed* — The beginning of foraging was defined by the first time a followed individual attempted to feed.

A period of increased movement at dawn was not discernible. It could have been easily masked by the activities of speedup, group formation, and initiation of feeding. I was also unable to determine times of cessation of activity of nocturnal fishes because of their relative sensitivity to diver presence at dawn.

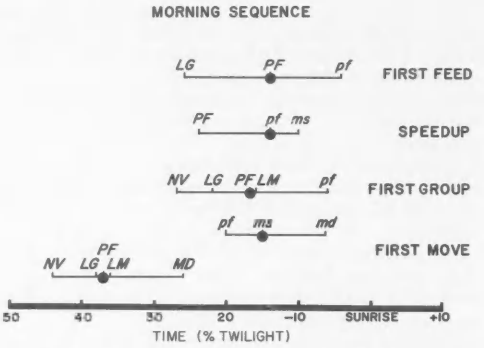


FIG. 2. Sequence of events in the Cazenovia Lake fish community during morning twilight as a function of time relative to sunrise. Species are abbreviated as in Table 1; capital letters are for PYOY, lower case for YOY. See Table 3 for details.

## ACCOUNTS OF ACTIVITIES OF INDIVIDUAL SPECIES

Following are details of twilight activities for eight diurnally active and six nocturnally active species. Time and light correlates of evening and morning activities of diurnal fishes are presented in Table 2, of nocturnal species in Table 3. Temporal designations are based on the activity patterns (= major feeding period) of nonbreeding adults and large juveniles, except where noted. General distribution and activity patterns are outlined as background for comparing twilight events. Day versus night and seasonal distribution patterns are detailed in Helfman (1979a).

The order of data presentation is (1) diurnal activity and habitat, (2) evening twilight activities, (3) nocturnal activity and habitat, (4) morning twilight activities, and (5) additional observations and reference to pertinent literature. Where ontogenetic differences were observed, younger fish are discussed first.

*Diurnal species (Table 2) — (1) Chain pickerel, Esox niger (Table 2a)* — In Cazenovia, this solitary predator foraged near or among deeper macrophyte beds (1.6–3.5 m). Nighttime distribution implies a dusk movement into shallow water: 48% of nocturnal sightings were in shoreline quadrats whereas only 24% of daytime sightings were near shore ($n$ = 52 and 109 sightings, respectively). A 30-cm untagged individual was observed on five separate evenings during 1 mo at the same shoreline locale, suggesting night-to-night resting site constancy. Comparative findings on twilight activities of chain pickerel are unavailable (e.g. Crossman and Lewis 1973). Scott and Crossman (1973) also imply an inshore evening movement, based on shallower nocturnal distribution.

*2) Mimic shiner, Notropis volucellus (Table 2c, k)* — This minnow formed diurnally foraging schools in 10–200 cm of

TABLE 2. Evening and morning twilight activities of diurnally active fishes in Cazenovia Lake, NY. Data are presented as "median, earliest, latest (*n*)" for time, "median, greatest, least (*n*)" for light values. Medians and ranges are used because intraspecific variation precludes presentation of "average" species characteristics. Minus (−) values are before, plus (+) after sunset or sunrise. SS = sunset, YOY = young-of-year, PYOY = post-young-of-year. All data are for PYOY unless otherwise indicated.

| | Evening time (% astronomical twilight) | | | | |
|---|---|---|---|---|---|
| Species | Increased movement | Last feed | Group breakup | First stop | Final stop |
| a) Chain pickerel | −1(1) | | | +24(1) | +32,+17,+38(7) |
| b) Golden shiner YOY | −1,−16,+11(4) | +16,+11,+22(2) | +10,+4,+17(4) | +26,+23,+30(2) | +31,+17,+38(4) |
| c) Mimic shiner | −2,−13,+5(6) | | | +28,+7,+35(4) | +29,+23,+47(4) |
| d) Bluntnose minnow[a] | | +19,+19,+19(2) | | +28,+22,+34(2) | +40,+38,+42(2) |
| e) Banded killifish | | +8,+6,+10(2) | +6,+5,+7(2) | +15(1) | |
| f) Pumpkinseed | +10,−8,+14(4) | +27,+10,+37(6) | | +34,+15,+34(3) | +36,+31,+55(7) |
| g) Bluegill[b] | +1,−8,+14(9) | | +16,+14,+18(5) | | +34,+16,+56(7) |
| h) Smallmouth bass | | | | | |
| YOY[a] | | −5,−7,+1(3) | | +19,+10,+27(5) | +30,+2,+38(8) |
| PYOY[c] | +5,+4,+8(3) | | | | +36,+27,+53(6) |
| i) Yellow perch | | | | | |
| YOY | −7(1) | −7,−8,+2(3) | +10,−1,+14(4) | +10,−8,+16(5) | +17,+14,+23(3) |
| PYOY | +8,−9,+14(10) | +5,+2,+13(5) | +16,+10,+21(4) | +18,+7,+32(13) | +25,+15,+40(18) |
| j) Largemouth bass YOY[a] | | | | +12,+6,+17(4) | +28,SS,+38(9) |

| | Morning time (% astronomical twilight) | | | | |
|---|---|---|---|---|---|
| Species | First move | Speedup | First group | First feed | Increased movement |
| k) Mimic shiner | −44,−52,−20(3) | | −27,−33,−20(2) | | −15,−24,−11(3) |
| l) Pumpkinseed | −38,−52,−34(10) | | −22,−39,−5(6) | −26,−38,−5(7) | |
| m) Bluegill[d] | −36,−47,−23(12) | | −16,−18,−4(3) | | |
| n) Smallmouth bass | | | | | |
| YOY[e] | −6,−9,−2(2) | | | | |
| PYOY[c] | −26,−35,−17(5) | | | | |
| o) Yellow perch | | | | | |
| YOY | −20,−28,−7(4) | −14,−19,−7(3) | −6,−19,−4(4) | −4,−4,−4(3) | |
| PYOY | −37,−56,−21(6) | −24,−34,−15(10) | −17,−23,−15(5) | −14,−24,+10(5) | |
| p) Largemouth bass YOY | −15,−15,−9(3) | −10,−11,−9(2) | −15,−15,−7(3) | | |

| | Evening light (lx) | | | | |
|---|---|---|---|---|---|
| Species | Increased movement | Last feed | Group breakup | First stop | Final stop |
| a) Chain pickerel | | | | | 0.16,1.8,0.005(7) |
| b) Golden shiner YOY | 93.9,232,38.0(4) | 4.1,38.0,0.45(2) | 9.4,57.3,6.2(4) | 0.28,1.7,0.05(2) | 0.005,0.13,0.002(4) |
| c) Mimic shiner | 181,862,93.9(6) | | | 0.05,2.2,0.01(4) | 0.001,0.02,$10^{-4}$(4) |
| d) Bluntnose minnow[a] | | 5.3,27.4,1.1(2) | | 0.06,0.28,0.02(2) | 0.001,0.003,$10^{-4}$(2) |
| e) Banded killifish | | 44.8,62.3,32.3(2) | 52.8,67.6,41.3(2) | 15.4(1) | |
| f) Pumpkinseed | 27.4,731,16.7(4) | 0.25,41.3,0.003(6) | | 0.07,15.4,0.02(3) | 0.005,0.03,$10^{-4}$(7) |
| g) Bluegill[b] | 52.8,731,1.4(9) | | 5.3,8.0,3.2(5) | | 0.003,0.42,$10^{-8}$(7) |
| h) Smallmouth bass | | | | | |
| YOY[a] | | 102,196,25.2(3) | | 1.1,16.7,0.23(5) | 0.07,27.4,$10^{-4}$(8) |
| PYOY[c] | | | | | 0.20(1) |

TABLE 2. (*Concluded*)

| Species | Evening light (lx) | | | | |
|---|---|---|---|---|---|
| | Increased movement | Last feed | Group breakup | First stop | Final stop |
| i) Yellow perch | | | | | |
| YOY | 673(1) | 322,379,101(3) | 18.2,93.9,7.4(4) | 16.7,322,5.3(5) | 4.5,7.4,0.08(3) |
| PYOY | 38.0,731,16.7(9) | 44.8,130,5.3(5) | 9.4,19.7,1.8(4) | 1.4,35.0,0.03(12) | $0.01,2.1,10^{-4}$(17) |
| j) Largemouth bass YOY[a] | | | | 12.0,16.7,1.8(4) | 0.04,110,0.001(9) |

| Species | Morning light (lx) | | | | |
|---|---|---|---|---|---|
| | First move | Speedup | First group | First feed | Increased movement |
| k) Mimic shiner | $0.03,10^{-5}$,0.06(3) | | 0.13,0.06,0.28(2) | 44.8(1) | 14.2,0.25,52.8(3) |
| l) Pumpkinseed | $0.002,10^{-6}$,0.32(10) | | 0.63,0.002,67.6(6) | $0.95,10^{-4}$,67.6(7) | |
| m) Bluegill | $0.004,10^{-6}$,0.68(12) | | 11.1,2.5,19.7(3) | | |
| n) Smallmouth bass YOY | 9.4(1) | | | | |
| o) Yellow perch | | | | | |
| YOY | 1.5,0.07,4.1(4) | 4.5,4.1,13.1(3) | 21.4,4.1,110(4) | 110,110,120(3) | |
| PYOY | $0.002,10^{-6}$,3.2(6) | 0.63,0.16,8.0(10) | 0.95,0.16,7.4(5) | 57.3,0.80,620(5) | |
| p) Largemouth bass YOY | 2.1,0.12,2.7(3) | 6.2,1.4,25.2(2) | | | |

[a]Includes aquarium observations on the Cazenovia Lake shoreline.
[b]Group breakup data for bluegill from aggregations not associated with floats.
[c]Includes data from Skaneateles and Mirror lakes.
[d]Group formation data for bluegill from aggregations not associated with floats.
[e]Includes data from Mirror Lake.

water. Shortly before sunset, schools engaged in a distinctive migration from vegetated shallows to the barren region at 6 m (= "increased movement" in Table 2; see also Black 1945). They arrived in this area in small groups, swimming 10–15 cm above the bottom. They then went through slowdown, ending in final stop 1–2 cm above the bottom. At night, fish were scattered along the bottom at densities $\leq 10/m^2$. In the morning they resumed activity by moving slowly 2–3 cm above the bottom, joining quickly with other individuals, and migrating to shallow water. Activities in the morning, particularly migration, occurred earlier and at lower light levels than corresponding activities in the evening. Not all individuals migrated at dusk; 6% of 815 fish sighted at night were in water <2.5 m deep. A few fish usually rested at 10- to 30-cm depths over small rocks or gravel. These fish may have been members of small schools seen in these areas by day (two such individuals were followed through dusk). Such individual variation is consistent with Hobson's (1972) findings that some members of twilight-migratory coral reef species typically do not take part in twilight migrations.

*3) Bluntnose minnow, Pimephales notatus (Table 2d)* — Individuals and small groups of bluntnose minnow occurred in very shallow rocky areas, both by day and night (87 of 91 day and 3 of 4 night sightings in ≤1.0 m water). Evening changeover, observed once in the field and once in a 100-L outdoor aquarium, included last feed, slowdown, and final stop. In Ontario, bluntnose minnow also occupied rocky habitats day and night (Emery 1973a). Although a diurnal forager, most spawning occurs at night (Westman 1938). Such a "breakdown" in diel activity patterns during breeding occurred in several other Cazenovia species (see accounts for pumpkinseed, yellow perch, brown bullhead, rock bass, largemouth). Other information on twilight activities is unavailable.

*4) Banded killifish, Fundulus diaphanus (Table 2e)* — Killifish formed bottom-foraging schools of 5–15 individuals in 0.6- to 1.6-m depths (50 schools, 429 fish), with 80% of all fish occurring in 1.0–1.6 m. At twilight, schools broke up, individuals stopped feeding, followed by slowdown and a series of 15- to 20-s stops. Most nighttime sightings (83% of 23 fish) where in <0.6 m, suggesting a slight shoreward movement at dusk. At night, killifish rested singly near the bottom. Published accounts similarly indicate exclusively diurnal feeding (Keast and Welsh 1968). Comparative findings on twilight activities are unavailable.

*5) Pumpkinseed sunfish, Lepomis gibbosus (Table 2f, l)* — By day, individuals or small groups foraged at most depths. The evening transition to inactivity was relatively rapid (Table 2). Increased movement was predominantly inshore by groups of smaller (<15 cm) individuals or offshore by larger individual fish. Fish often defended a resting site, particularly against other small pumpkinseed (see below). Some pumpkinseed were active relatively late (Table 2), including feeding until SS + 33, nest guarding until SS + 37, courtship until SS + 46, and nest fanning at SS + 66. Resting fish were easily aroused, as evidenced by three individuals that picked at zooplankton illuminated in a flashlight beam. Resting site constancy was not evident on successive nights. Published accounts indicate a dusk vertical movement toward the substrate with nocturnal resting in contact with the bottom, usually between rocks or near fallen logs (Emery 1973a). Cazenovia Lake pumpkinseed showed little nocturnal attraction to such structure.

*6) Bluegill sunfish, Lepomis macrochirus (Table 2g, m)* — Most data on bluegill in Cazenovia refer to the abundant individuals <13 cm. By day, these fish occurred in three areas of the study site. (A) In shallow (<1.6 m) water they foraged as individuals in the upper layers of short vegetation. (B) Aggregations of up to 300 fish occupied moderate depths (1.6–2.5 m) over vegetation or under floats (Helfman 1981); these fish fed occasionally from plant and float surfaces or in the water column. (C) Zooplanktivorous bluegill occurred with yellow perch and pumpkinseed over deeper water (3.25–6.0 m). At twilight, medium-depth and deep-water aggregations broke up, individuals assumed nighttime coloration (darkened median and pelvic fins), and fish moved to shallower water (= increased movement). Many fish then stopped just above the bottom in 1.6–2.5 m of water, although these fish were easily disturbed by diver activity. At dawn, activity resumed relatively early (see Table 2). Float-associated and deep-water aggregations reformed considerably after activity commenced. Large (>17 cm) bluegill behaved very differently. These fish frequented the margins and tops of the heavily vegetated drop-off region (3.25–6.0 m) by day and commonly continued activity well into the night. Nest-guarding males fed as late as SS + 50 and group spawning occurred as late as midnight (W. C. Dominey, Cornell Univ., personal communication). Large individuals moved about slowly in the dark and fled quickly when illuminated. Published accounts of activity patterns in bluegill indicate variable behavior. Laboratory investigations show a swimming peak at dawn (e.g. Davis 1964) whereas field studies show a feeding peak or inshore movement at sunset and an offshore movement at dawn (Keast and Welsh 1968; Baumann and Kitchell 1974)). These movements also vary as a function of fish size (e.g. Werner 1969; Werner et al. 1977). In general, some activity and feeding occurs throughout the diel cycle, particularly during the day.

*7) Smallmouth bass, Micropterus dolomieui (Table 2h, n)* — YOY smallmouth in Cazenovia were territorial throughout the day. In both the field and a 100-L outdoor aquarium, fish chased other small conspecifics after feeding ceased and prior to developing a blotched coloration typical of all resting smallmouth (see Emery 1973a). Adult smallmouth were uncommon in Cazenovia, particularly at depths of <5 m (mean daytime density along transects in 1976 = 0.004 fish/$m^2$, all 48 sightings in 3.25–7.5 m). Smallmouth in Skaneateles and Mirror lakes (Table 2) behaved as described by Emery (1973a), whose data suggest an offshore evening movement, breakup of aggregations, slowdown, and final stop. Final feeding may have occurred during slowdown. In Skaneateles and Mirror lakes, large individuals (>30 cm) were active later in the evening and earlier in the morning than were smaller conspecifics, suggesting an increase in total activity time with size. At night, all sizes rested near the bottom, with YOY preferring small overhangs or cracks in large rocks. Although smallmouth bass are caught by anglers at night, most studies suggest inactivity after dark (e.g. Munther 1970). Several studies showed feeding or activity peaks during crepuscular periods (e.g. Hubbs and Bailey 1938; Emery

TABLE 3. Evening and morning twilight activities of nocturnally active fishes in Cazenovia Lake, NY. Data are presented as "median, earliest, latest (*n*)" for time, "median, greatest, least (*n*)" for light values. Minus (−) values are before, plus (+) values after sunset or sunrise. All data for post-young-of-year.

| | Evening time (% astronomical twilight) | | | Evening light (lx) | | |
|---|---|---|---|---|---|---|
| | Group breakup[d] | First active | Increased movement | Group breakup | First active | Increased movement |
| a) Golden shiner PYOY | | | +12,+9,+20(6) | | | 13.1,29.7,5.8(5) |
| b) Brown bullhead,[a] | | | | | | |
| visibility >3 m | | +29,+9,+40(15) | | | 0.16,73.4,0.002(15) | |
| visibility <3 m | | −1,−6,+4(3) | | | 21.4,73.4,16.7(3) | |
| c) Rock bass[b] | | | | | | |
| >5 cm | +16,+5,+40(6) | | | | | |
| <5 cm | +32(1) | | | | | |
| d) Largemouth bass PYOY | | +42(1) | | | 1.5(1) | |
| e) Black crappie | −6,−90,+14(4) | | +1,−24,+12(8) | 411,5245,11.1(4) | | 21.4,862,0.28(8) |
| f) Walleye | | +41,+29,+46(5) | | | 0.002,0.04,$10^{-5}$(5) | |
| | Morning time (% astronomical twilight) | | | Morning light (lx) | | |
| | Group formation | Cease activity | Migration | Group formation | Cease activity | Migration |
| g) Brown bullhead[c] | | −38,−45,−31(2) | | | 8.0(1) | |
| h) Rock bass[b] >5 cm | −13,−22,+4(7) | | | | | |
| i) Black crappie | +41,−8,+101(3) | | −1(1) | 6711,73.4,7909(3)[e] | | 38.0(1) |

[a]Nonnesting season values only: during nesting season (June–early July) time = SS, −27,+19(10); light = 101,2505,0.08(10).
[b]Skaneateles Lake, NY, data only.
[c]Includes data from Mirror Lake, NH.
[d]First active for species that formed diurnal resting groups.
[e]Black crappie light levels are median, least, greatest.

1973a).

*8) Yellow perch, Perca flavescens (Table 2i, o)* — Twilight activities of yellow perch were detailed in Helfman (1979b). Briefly, perch are diurnal except for nocturnal spawning. At dusk, they engage in increased movement (= "flux"), last feed, group breakup, slowdown, and final stop. Dawn activities mirrored the evening sequence but without any definable increased movement. Schools of YOY perch changed over at higher light levels both evening and morning than larger conspecifics. Twilight activities of individuals were highly synchronized within the schools of young perch and asynchronous in older fish. Differences between age groups may have resulted from greater predation on smaller fish. Literature comparisons indicate diurnal activity, nocturnal inactivity, and considerable variability both between and within populations with respect to twilight activities.

*Nocturnal species (Table 3) — (1) Golden shiner, Notemigonus chrysoleucas (Tables 3a, also 2b)* — YOY golden shiner fed by day. In mid-June, schools of 10-mm fish drifted 1 m from shore and ceased activity at dusk. YOY golden shiner were active later and coordination of twilight activities within schools decreased as fish grew. YOY were immobile at night in <1 m, just below the water surface. By day, adult shiners formed active schools near deep macrophyte beds and wooden floats. Adult fish were active later than YOY. At night, some fish occurred individually near the shoreline (16 of 35 night sightings were in shoreline quadrats), suggesting an evening inshore movement. No comparable data are available for YOY *Notemigonus*. Zaret and Suffern (1976) found daytime schools of adult shiners in the littoral zone of a Connecticut pond that migrated to open water considerably earlier (at SS − 2.5 h) than in Cazenovia. Golden shiner in a small Michigan Lake schooled by day in the littoral zone; ~80% of the population moved offshore at twilight (SS + 30 to 45 min) to feed near the surface on zooplankton until midnight (Hall et al. 1979). The fish I encountered in shallow water probably represented only part of the population, the remaining fish moving offshore.

*2) Brown bullhead, Ictalurus nebulosus (Table 3b, g)* — Brown bullhead are nocturnal foragers (e.g. Keast and Harker 1977). During the nonbreeding season, bullhead rested by day in bottom vegetation at depths of 1–4 m and had to be prodded before swimming off. On evenings when visibility was <3 m, fish initiated activity relatively early and at high light levels (Table 3b). At night, solitary fish moved actively just above vegetation; they swam off rapidly when illuminated. Bullhead had more variable activity times during the hole-nesting phase, with less apparent sensitivity to turbidity (Table 3b). Nest-guarding adults swam toward divers by day and ignored a flashlight beam at night. Variability in activity periods and a tendency toward more diurnal activity during reduced light (= turbid?) conditions have also been shown in laboratory experiments (Eriksson 1978). Comparable findings on twilight activities are unavailable.

*3) Rock bass, Ambloplites rupestris (Table 3c, h)* — In Skaneateles Lake, daytime groups of up to 17 large (>8 cm) rock bass hovered near logs and other objects in 3–8 m. These groups were probably daytime resting schools, similar to nocturnal species on coral reefs (e.g. Hobson 1968). During the half hour after sunset, individuals or small groups left the aggregation and moved into shallow water, where they occurred individually. In the morning, fish arrived at the daytime resting area during the half hour prior to sunrise. Large rock bass in Cazenovia formed daytime resting groups of only two to five individuals near tall *Ceratophyllum* in 3.25–6.0-m depths. Large individuals generally avoided shallow areas by day except during the breeding season. I followed shallow-dwelling fish on five evenings and six mornings and could not distinguish definitive twilight changeover activities. At night, rock bass were seen commonly in most areas of the study site, particularly during early spring and late fall (Helfman 1979a). Rock bass show a mixture of diurnal and nocturnal activity (Keast and Welsh 1968; Emery 1973a). For the present discussion, this benthic predator is considered nocturnal, but with considerable daytime and crepuscular activity and individual variation.

*4) Largemouth bass, Micropterus salmoides (Tables 3d, also 2j, p)* — YOY largemouth were diurnally active. Schools of guarded young foraged in <1.5 m of water. At twilight, these fish slowed down and then lost parallel orientation. All fish in a school ceased activity in <10 min without dispersing. At night, YOY rested in macrophytes in <1.0 m of water. At dawn, neighboring individuals began movement, initiated parallel swimming, and schools reformed and began moving. Parental guarding ended when YOY were 30–35 mm long; young fish then occurred as individuals or in small groups (fewer than six fish) at all depths with dense vegetation. They often associated with large schools of golden shiner YOY on which they fed. Dusk changeover differed from guarded young, with one or two short stops rather than slowdown preceding final stop. Individual variation increased, with final stop differing by as much as 30 min between fish only 3 m apart. Resting sites were still in leaf axils in dense vegetation. Intermediate size largemouth (6–20 cm) were also diurnally active, foraging at all depths as individuals or in groups of up to 50 fish. Changeover and nocturnal activity data are unavailable. Three individuals were inactive after dark for periods of 3 min, 9 min, and 5 h. Adult largemouth (>20 cm) in Cazenovia Lake avoided divers, particularly after the breeding season (June through early July). They occurred at most depths and were associated with dense vegetation, other physical structure, and abundant prey. Nine melanistic individuals lay motionless on the bottom in short vegetation in 1 m for up to 1.5 h. Their inactivity and the low abundance of prey fish in this area (least populated of nine habitat zones) suggest that these fish were resting and not lying in wait for prey. Once such fish became active at SS + 42, the only changeover data I have for nonbreeding adults. Two adult males that guarded free-swimming young by day left their broods at SS − 20 and SS − 2. The same two fish returned the following mornings at SR − 23 and SR − 5, respectively. During observations of the SS − 2/SR − 5 male, predation on the unguarded young by bluegill, pumpkinseed, and rock bass was intense. Attacks increased to 80/min during the 25 min after the parent departed, and fell from 100/min to 18/min within 15 min of the parent's return the next morning. Largemouth are considered both diurnal and nocturnal feeders (e.g. Heidinger 1975), although larger bass are apparently more willing to take lures at night. Boyer and Hirsch (in Miller 1975) reported both juvenile and

adult largemouth resting at night in a quarry in Kansas. Feeding activity may peak during crepuscular periods (Zweiacker and Summerfelt 1973), although data from angling success should be interpreted with caution (see Heidinger 1975). Comparative findings on twilight activities of any size-class of this important predator are unavailable.

*5) Black crappie, Pomoxis nigromaculatus (Table 3e, i)* — These primarily nocturnal planktivores formed small daytime resting aggregations near anchored floats or in the water column in deep macrophyte beds. Small individuals (<15 cm) fed occasionally in the afternoon whereas larger individuals were not seen feeding by day. Changeover was size dependent. Individuals <15 cm departed from groups later on three evenings than did larger conspecifics; on three other occasions progressively smaller fish moved past a deep-water (5–7 m) locale. The data (Table 3) suggest that movements between resting and feeding areas occurred as light intensity began to decline rapidly in the evening or began to level off in the morning. At night, crappie occurred singly both deeper and shallower than the daytime locales. Similar day/night patterns have been observed in other North American lakes (Keast and Harker 1977; Werner et al. 1977). Comparative findings on twilight activities are unavailable.

*6) Walleye, Stizostedion v. vitreum (Table 3f)* — Only 11 walleye were seen in the Cazenovia study area during this investigation. By day, individuals swam 1–2 m off the bottom at 4 m or greater depth, although three 40- to 60-cm fish were seen resting motionless on the bottom in the vegetated drop-off region. In the evening, walleye were last seen in deep water shortly after sunset and were first seen in shallow (0.5–1.5 m) regions a half hour later. These observations are in close agreement with activity times obtained by ultrasonic tracking (Kelso 1978). Other observational studies (Ryder 1977), as well as retinal histology (Ali et al. 1977), indicate that walleye forage most successfully during crepuscular periods and at night. Periods of activity change, however, as a function of water clarity (Ryder 1977).

## Other Fish Activities at Twilight

*Agonistic behavior* — Aggressive interactions peaked shortly after sunset (Fig. 3). These interactions, excluding those of breeding individuals, were seen during 13% of dusk dives but only during 3% of dawn and 5% of daytime dives. Most acts were by small centrarchids, particularly bluegill and pumpkinseed, that chased passing fishes away from resting sites (see also Hobson 1972; Ehrlich et al. 1977; but see Robertson and Sheldon 1979).

Attacks by nest-guarding male centrarchids were similarly maximal between sunset and SS + 20 and then fell rapidly (Fig. 4). This peak corresponds to the general period of increased movement, when numerous passing fish would have been encountered. The fall-off reflects the general lack of nighttime fish activity in the shallows, although nest-guarding centrarchids actively fanned and guarded long after other fishes stopped moving through the area.

Similar trends are suggested at dawn (Fig. 4b). Aggression increased after SR − 30, as did activity of most diurnal fishes. The subsequent morning decrease in agonism could have resulted from the movement of many diurnal species to deeper water.

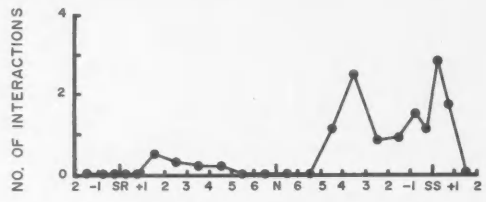


FIG. 3. Agonistic interactions relative to time of day in the Cazenovia Lake fish community. The frequency of interactions per hour of observation is shown, not including interactions associated with nest-guarding or courtship. Peak at SS − 3 is largely the result of defense of an apparent feeding territory by a single 8-cm pumpkinseed on one afternoon. Total observation time = 98 h, total interactions = 88.

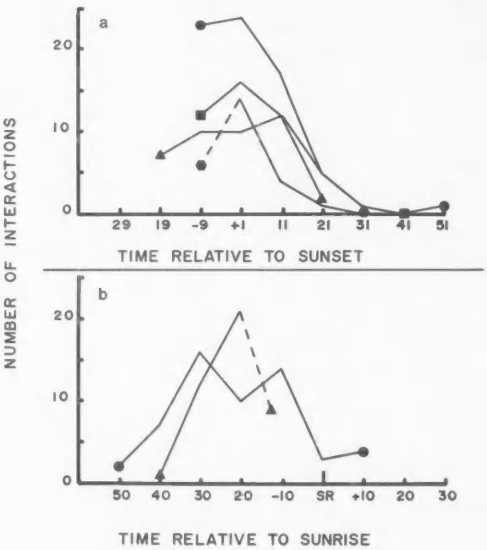


FIG. 4. (a) Chasing frequencies for nest-guarding male centrarchids at dusk. Data are from four individuals, taken over 10-min intervals. Data points are at the end of each 10-min interval. Total observation time = 4.3 h, total interactions = 183 (circles = 20-cm rock bass, squares = 38-cm largemouth bass, triangles = 20-cm pumpkinseed, hexagons = 17-cm bluegill). (b) Chasing frequencies for nest-guarding male centrarchids at dawn. Data are from two individuals, including the same 20-cm rock bass (circles) as in (a) above, observed the following morning. Data points are at the end of each 10-min interval. Dotted line for the 15-cm pumpkinseed (triangles) represents unknown values when chase frequency had qualitatively decreased. Total observation time = 2.3 h, total interactions = 90.

*Comfort activities (Fig. 5)* — Low levels of yawning, chaf-

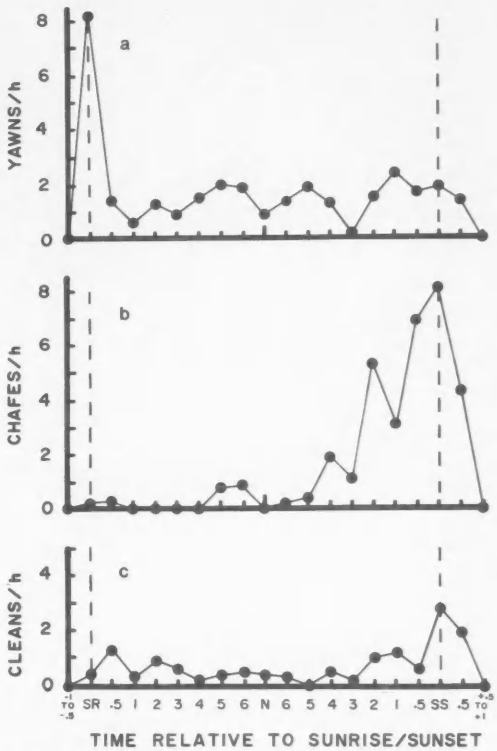


FIG. 5. Increase in three comfort behaviors at dusk and dawn in Cazenovia Lake fishes, 1975 and 1976. Data begin at SR − 1 (1 h before sunrise) and proceed to SS + 1 (1 h after sunset). Each value is for the hour preceding the time given on the abscissa, except for sunrise and sunset, which have been subdivided into half-hour periods to show twilight activities on a finer scale. N = noon. (a) Yawning frequency (totals: 157 yawns/92 observation hours). (b) Chafing frequency (totals: 190 chafes/92 observation hours). (c) Frequency of cleaning interactions. An interaction was defined as a complete or incomplete sequence involving pose, inspect, and clean (after Losey 1971). The data do not include 19 inspects by a 9-cm bluegill over a 13-min period; if these data are included, the value at SS − 5 becomes 2.9 interactions/h (totals: 81 interactions/107 observation hours, not including the 9-cm bluegill).

ing (rubbing against hard surfaces), and cleaning (picking at body and fins by small bluegill) were observed throughout the day in most species. Yawning occurred most frequently at dawn, usually shortly after initial movement. Chafing increased after noon and peaked just prior to sunset.

A morning peak in yawning was also observed in a diurnally active Hawaiian wrasse during morning twilight, shortly after leaving its nocturnal resting site (Hobson 1972). The evening increases in chafing and cleaning in Cazenovia suggest that these "comfort" activities (Baerends and Baerends-Van Roon 1950) may occur more readily after feeding ceases.

## General Discussion on A Comparison of Twilight Activities in Temperate and Tropical Fish Communities

Certain trends recur between and within Cazenovia Lake species that suggest general community characteristics and that have parallels in the better known fish communities of coral reefs (Table 4a). These patterns are (1) a sequence of twilight activities at dusk, repeated in reverse at dawn, and (2) between and within species differences in timing and behavior related to age and size. Other activities contrast with those of coral reef fishes (Table 4b), including (1) shelter use by resting fish, (2) twilight migrations, (3) time and light levels at changeover, and (4) variability in the timing of changeover activities. Taken together, similarities and differences in the patterns may indicate underlying determinants of temporal structure in the different assemblages.

### Similarities between Community Types

*Twilight sequence* — At dusk, individual diurnal lake fishes undergo a relatively ordered sequence of five behaviors (Fig. 1). At dawn most elements in the sequence are repeated but in reverse order (Fig. 2). The occurrence of these activities in most species results in a community sequence through twilight.

Although the two habitats differ greatly, a five-phase twilight sequence was also noted by Hobson (1972) in Hawaiian reef fishes (initial overt phase, upsurge in activity, descent of planktivores, evacuation of the water column, cover seeking). The two sequences are not strict analogs, but do contain parallel elements. Both initial overt stage and upsurge in activity involve group and individual movements between feeding and resting areas, paralleled by increased movement/migration of seven Cazenovia species. Descent of the planktivores has its analog in the dispersion of planktivorous schools of bluegill, pumpkinseed, and yellow perch, which moved down and into shallower water. Cover seeking may parallel cessation of activity, although Cazenovia Lake fishes sought cover less commonly (see below).

Other coral reef investigators noted a species sequence in the final event of evening changeover (cover seeking or disappearance of diurnal fishes). Studies of single species (e.g. Ogden and Buckman 1973; Robertson and Sheldon 1979) also found elements of Hobson's five-part sequence. These results imply that changeover in many fish communities will involve behaviors specific to twilight periods, performed at slightly different times by different fishes.

*Size and age-related differences* — Timing differences occurred between size-classes of Cazenovia Lake species (Fig. 1). Large golden shiner, bluegill, smallmouth bass, largemouth bass, and yellow perch were active later than small conspecifics; golden shiner, bluegill, and largemouth fed at night as adults. Ontogenetic differences in timing may result from greater predation pressure on smaller fish. During 300 dives in 3 yr, I witnessed only four attacks on PYOY fish (disregarding attacks on mimic shiners by largemouth bass). By comparison, attacks on YOY golden shiner, largemouth bass, and perch were commonplace, reaching as high as 15

TABLE 4. Similarities and differences in trends in the twilight activities of Cazenovia and other temperate lake fishes versus tropical marine species. Parenthetical references for tropical marine studies and other temperate communities are given in the footnote.

| |
|---|
| A) Similarities between both systems |
| 1) Progressive sequence of activities at dusk; reversed at dawn (6, 14, 36)[a] |
| 2) Dawn activities at lower light levels than at dusk; increased variability at dawn (1, 6, 12, 14, 21, 25) |
| 3) Upsurge in nonfeeding activities, particularly agonism associated with resting site defense at dusk (2, 5, 8, 14; see also 40) |
| 4) Ecological replacement sets, shifting to older individuals and larger-mouthed species at dusk (4, 7?, 9, 16, 18, 23, 39) |
| 5) Change from group to individual activities in both diurnal and nocturnal subcommunities at dusk (4, 9 (except *Osmerus mordax*), 10, 11, 12, 13, 17, 28, 39; but see 8, 30) |
| 6) Breakdown in characteristic twilight activities during breeding season (10, 19, 21, 22, 28, 33; see also 40) |
| 7) Upsurge in predation at twilight (3, 10, 11, 12, 13, 17, 19, 25, 28, 29, 31, 36) |
| 8) Earlier changeover in smaller individuals of a species and in smaller species at dusk, later at dawn (14, 28) |
| B) Differences in Cazenovia Lake fishes |
| 1) Less resting site constancy and structural affinity in diurnal fishes (compare with 4, 8, 14, 24, 34, 35, 37, 38, 39; see also 7) |
| 2) Fewer group migrating species (12, 15, 25, 27, 32, 33) |
| 3) Lack of a quiet or interim period (13, 14, 25, 28) |
| 4) Increased cleaning activity at dusk |
| 5) Increased intraspecific and ontogenetic variability in timing |
| 6) Later times and less light for most twilight activities of diurnal species at dusk (4, 6, 14, 28) |
| 7) More prolonged changeover |
| 8) Greater apparent between-species overlap in changeover time |

[a]References: 1, Blaxter 1976; 2, Bright 1972; 3, Childers and Shoemaker 1953; 4, Collette and Talbot 1972; 5, Cummings et al. 1966; 6, Domm and Domm 1973; 7, Ebeling and Bray 1976; 8, Ehrlich et al. 1977; 9, Emery 1973a; 10, Emery 1973b; 11, Fishelson et al. 1971; 12, Gladfelter 1979; 13, Hobson 1968; 14, Hobson 1972; 15, Hobson 1973; 16, Hobson 1974; 17, Hobson 1979; 18, Hobson and Chess 1976; 19, Hubbs and Bailey 1938; 20, Johannes 1978; 21, Kavanau and Peters 1976; 22, Lobel 1978; 23, Lowe-McConnell 1975; 24, Luckhurst and Luckhurst 1978; 25, Major 1977; 26, MacLean and Magnuson 1977; 27, McFarland et al. 1979; 28, McFarland and Helfman unpublished data; 29, Miller 1963; 30, Morin et al. 1975; 31, Munz and McFarland 1973; 32, Ogden and Buckman 1973; 33, Ogden and Ehrlich 1977; 34, Reinboth 1973; 35, Robertson and Sheldon 1979; 36, Ryder 1977; 37, Smith and Tyler 1972; 38, Sbikin 1977; 39, Starck and Davis 1966; 40, Woodhead 1966.

attacks per minute per predator during one morning twilight. Coordination of activities within polarized schools of young is probably an antipredator device (e.g. Hobson 1978). Such coordination was observed in Cazenovia during evening increased movement (mimic shiner, YOY golden shiner, YOY largemouth, YOY yellow perch), last feed (YOY perch, probably YOY largemouth), slowdown (YOY largemouth and YOY perch), and cessation of activity (YOY golden shiner, YOY largemouth, YOY perch) (see Fig. 1 and species accounts). Similar trends at dawn include relatively quick group formation in YOY largemouth and in small versus larger perch (see Fig. 2; Helfman 1979b; Helfman et al. 1982).

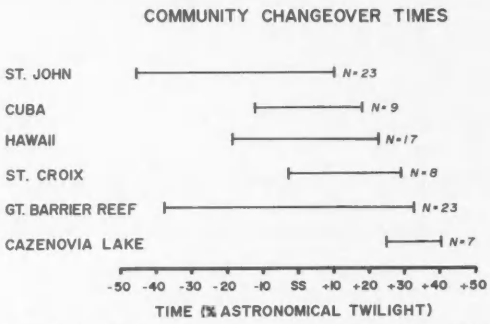


FIG. 6. Relative changeover times during dusk in five reef fish communities versus Cazenovia Lake, NY. Ranges are from values of diurnal species' means given by the various authors. Reef data are from Collette and Talbot (1972) for St. John, Virgin Islands; Sbikin (1977) for Gulf of Batabano, Cuba; Hobson (1972) for Kona, Hawaii; McFarland and Helfman (unpublished data) for St. Croix, Virgin Islands; and Domm and Domm (1973) for Hook Island, Great Barrier Reef. $N$ = number of species observed. All values have been converted to percent astronomical twilight.

The attraction of smaller fishes to plants and other structure may also be a defense against predation. This affinity was strong in young of golden shiner, smallmouth, largemouth, and perch, particularly at night. Similarly, if resting site choice affects predation, then resting site defense might be adaptive. Such defense was observed in juveniles but was uncommon in adult fishes after the breeding season.

Developmental differences in twilight activities were also noted by Hobson (1972), who found that smaller individuals and species initiated cover seeking earlier at dusk. He equated this trend to greater predation pressure on smaller fishes. The mechanism underlying such increased predation may be that the small eyes of small fishes capture less light, which prohibits continued activity during the diminishing light of twilight (Walls 1942; Munz and McFarland 1973; Manteifel et al. 1978).

## DIFFERENCES BETWEEN COMMUNITY TYPES

*Shelter seeking* — Most diurnal fishes on coral reefs enter shelter in the evening. Often, an individual uses the same shelter site repeatedly (Hobson 1972; Smith and Tyler 1972; Reinboth 1973; Ehrlich et al. 1977; Luckhurst and Luckhurst 1978; Robertson and Sheldon 1979). In Cazenovia, most fishes rested in relatively exposed locations, while resting site fidelity was low (Helfman 1979a). Ebeling and Bray (1976) found that temperate marine species do not seek cover at dusk whereas tropical derivative species in the same community do. Smith (1978) has suggested that coral reef fish communities may be organized around fish size, related to the size of the shelter hole into which a fish can fit. Shelter may be more important in coral reef than in temperate marine and lake fish communities.

*Migrations* — Many tropical reef species undergo twilight group migrations between feeding and resting areas, involving numerous fishes that take the same paths every evening and morning (Hobson 1973). I observed no such cor-

ridors of movement in Cazenovia. Only mimic shiner performed a definitive group migration, although yellow perch migrate in other lakes (Hasler and Bardach 1949). Nocturnally foraging black crappie and golden shiner in Cazenovia and rock bass in Skaneateles Lake dispersed at twilight. Similarly, Emery (1973a) described onshore twilight movements in three nocturnal lake species, and Bohl (1980) reported offshore twilight migrations in four nocturnally feeding cyprinids. Migrations by nocturnal fishes on coral reefs are particularly common and in this respect the two systems are similar. However, the relative occurrence of precise, distinctive migrations is apparently less in lakes than on coral reefs.

*Time, light, and the "quiet period"* — Changeover times in five tropical reef studies versus Cazenovia Lake are compared in Fig. 6. Changeover in Cazenovia occurred significantly later than in each coral reef assemblage (Mann-Whitney *U*-tests, Siegel 1956, $P < 0.01$). For the two tropical studies that used similar light measuring techniques (Hobson 1972; W. N. McFarland and G. S. Helfman, Cornell University, unpublished data), diurnal Cazenovia fishes changed over at significantly lower light levels. One aspect of these apparent timing differences is a missing "quiet period" in Cazenovia. This occurs when the water column is relatively devoid of small fishes (Hobson 1972). It happens in the tropics between ~15 and 35 min after sunset (~20–45% twilight), when light falls from 10 to 0.01 lx. It is also the interval between the settling down of diurnal fishes and the emergence of nocturnal foragers. In Cazenovia, many fishes were still in the water column at this time (Fig. 1). Most notably, the activity periods of nocturnal and diurnal fishes in Cazenovia overlapped considerably.

*Variability in timing* — In contrast with coral reefs, changeover timing in Cazenovia was imprecise (e.g. Hobson 1972; Sbikin 1977; McFarland et al. 1979). This is obvious to an observer but is difficult to quantify, in part because Cazenovia Lake fishes roam over extensive areas during twilight and show little resting site constancy.

Although data from the two community types were not collected identically, I have compared the average range of times for species changeover, contrasting time of last individual seen (four tropical studies) versus time of final stop (Cazenovia). The lake species have significantly greater ranges in this behavior than do the coral reef fishes studied by Hobson (1972), Domm and Domm (1973), and McFarland and Helfman (unpublished data) (Mann-Whitney *U*-tests, $P < 0.01$). Variability was not greater than for the St. John study of Collette and Talbot (1972).

Increased variability in Cazenovia may have resulted from prolongation of the final phases of changeover. Many tropical reef fishes enter shelter shortly after feeding ends (Hobson 1972; Ehrlich et al. 1977). Cazenovia fishes ceased feeding and then behaved agonistically, chafed, or engaged in cleaning prior to final stop (Fig. 1, 3–5). Cleaning is unlikely at this time on coral reefs as many cleaners are wrasses, which are among the first species to retire in the evening. Bluegill, however, were active late in twilight and were therefore available to clean other fishes.

Another indication of twilight variability may be the relative diurnality or nocturnality of species in a community. Hobson (1974) found that 80 of 114 species in Kona were predominantly either diurnal or nocturnal. Goldman and Talbot (1976, p. 141) also refer to "clear differences" in changeover activities of diurnal and nocturnal coral reef species. In contrast, Emery (1973a) found that only 4 of 20 temperate lake species were definitively diurnal or nocturnal; in the other 16 species, active and inactive members could be encountered simultaneously during at least one period of the diel cycle. Similarly, I found mixed temporal patterns in 12 of 18 Cazenovia species. Ebeling and Bray (1976), comparing diel patterns in temperate kelp bed versus coral reef fishes, concluded that "the kelp-bed community is the more loosely 'programmed' " (p. 715). Such observations suggest a trend toward greater temporal specialization in tropical reef fishes.

## Possible Determinants of Twilight Activity and Changeover

Coral reefs and temperate lakes differ in at least five ecologically influential factors: species diversity, predator density, water clarity, twilight length, and seasonal and climatic instability. These factors may interact and contribute to the above-mentioned differences in twilight activities in the systems.

*Species diversity and predator density* — Coral reef fishes typically seek shelter during inactive periods. If this is a predator avoidance behavior, and if shelter availability is potentially limiting, then prey may compete for hiding places. Such competition is more likely in the highly diverse communities that inhabit coral reefs (Helfman 1978; Robertson and Sheldon 1979). This competition could be reduced by slight differences in the shelter requirements of different species (e.g. Smith and Tyler 1972; Luckhurst and Luckhurst 1978), thereby narrowing the spatial niche dimensions of species in a community. By analogy, crepuscular predators may be a selective force affecting the breadth of temporal niche characteristics of prey fishes. Narrowing of the temporal niche could include a reduction in variability of twilight activities. Coral reef fishes might be expected to show less twilight variability than fishes subjected to less intense predation pressure.

Predators, whose activities peak during crepuscular periods in most fish communities, were relatively sparse in the Cazenovia study site (total density of four predator species = $0.014/m^2$, see Table 1). By comparison, Chave and Eckert (1974) listed 36 piscivorous species among 214 fishes at Fanning Island. Nine piscivores were common at one or more sites, reaching densities in excess of $0.04/m^2$ per species. A single predator, the graysby (*Petrometapon cruentatum*), was present at densities of up to $0.1/m^2$ on coral reefs in Curaçao (Nagelkerken 1977). In fact, large predators are relatively uncommon in many temperate freshwater fish communities, and prey fishes may quickly reach a body size refuge from predation (see Paine 1976). Relaxed crepuscular predation might lead to increased variability in twilight behaviors of larger prey fishes in Cazenovia.

It is widely held that selection has strongly favored coral reef fishes with effective predator avoidance behaviors. Even in Hawaii, where certain important predator groups are lacking for biogeographic reasons, the well-defined sequence of twilight behaviors has been attributed by Hobson (1972) to the threat of crepuscular predators. In considering the general absence of large piscivores on Hawaiian reefs, compared with

many other tropical reefs, Hobson suggested that the twilight behaviors there reflect an "historic threat" from predators (p. 738). His position is that these twilight behaviors originated elsewhere in the ancestors of present-day Hawaiian fishes and persist on Hawaiian reefs even though the predation pressures that originally led to their selection may now be reduced.

In contrast, temperate lakes and ponds often contain very low species diversities and are created, altered, and destroyed on a time scale much shorter than for coral reefs. Consequently, temperate fishes may have frequently existed both in the presence and absence of crepuscular predators. Such a varied history could presumably lead to the evolution of individuals that are able to alter their behavior to maintain activity relatively late into the crepuscular period when predators are uncommon or absent.

*Water clarity and twilight length* — Crepuscular predators on reefs have visual specializations and foraging techniques for hunting during twilight periods of rapidly changing and diminishing light (Hobson 1968; Munz and McFarland 1973). With the possible exception of the walleye (Ali et al. 1977), temperate freshwater predators do not show similar specializations. Crepuscular predators rely heavily on an ability to strike at backlit prey from below (Hobson 1966). The depth over which this advantage functions would be greatly reduced in turbid water. The frequent or unpredictably poor visibility characteristic of temperate lakes could therefore reduce any visual advantage a specialized crepuscular predator might have (see Lythgoe 1979).

The duration of twilight activities may be directly related to twilight length. Temperate cyprinids migrate more rapidly during periods of shorter twilight (Bohl 1980). The swarming times of mosquitoes also vary as a function of latitude, with longer swarming times during longer twilight (Nielsen and Nielsen 1953). Similarly, evening courtship activities of American woodcock (*Philohela minor*) are prolonged at higher latitudes, perhaps due to longer twilight (Wishart and Bider 1977). A related phenomenon may affect temperate lake/tropical reef comparisons. Twilight behavior may be directly related to and limited by the speed of photomechanical movements involved during dark adaptation of the fish eye (Ali and Hoar 1959; Munz and McFarland 1973; McFarland et al. 1979). Light in the tropics could be changing at a rate that approaches the rate at which the fish eye adapts. Twilight is much longer at higher latitudes (94–144 min in Cazenovia versus 72–84 min in the coral reef studies) and the rate of change of light might be sufficiently slow to allow for some variability in twilight activities.

*Seasonal and climatic instability* — Climatic stability in tropical regions is relatively constant compared with temperate conditions (e.g. MacArthur 1972). Temperate lake fishes occur in seasonally varying habitats that become marginal for fish existence in terms of both winter and summer environmental extremes of temperature, oxygen, and food availability. Temperate fishes have evolved in a geographic region that has undergone repeated glaciations, and under conditions of varying species diversities and community compositions. Such an evolutionary history would favor much less specilization than is the case on coral reefs, where relative stability at this level apparently correlates with greater diversity, and greater diversity in turn may result in increased specialization (MacArthur 1975; but see Sale 1977). Many lake species are exceedingly plastic in their habitat and feeding preferences (e.g. Larkin 1956; Keast 1978; Werner and Hall 1979; Helfman 1979a, b). Such a generalist strategy might also apply to temporal habits.

The foregoing discussion suggests that, as part of a general ecological plasticity, temperate lake fishes might be expected to vary their temporal characteristics as a function of the interactions of community diversity, water clarity, predator abundance, and twilight length. All of these factors occur in various combinations in the lakes, springs, and ponds of North America, many of which have species in common (e.g. bluegill occur from southeastern Canada to the Florida Everglades, yellow perch occur from Great Slave Lake, Northwest Territories, to the Florida Panhandle; Scott and Crossman 1973). A working hypothesis, therefore, would be that the twilight activities of freshwater species will converge toward those of coral reef fishes as species diversity, water clarity, twilight length, and crepuscular predation levels more closely resemble those found in the coral reef environment.

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# The Effect of Phosphorus Limitation on Algal Growth Rates: Evidence from Alkaline Phosphatase[1]

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SMITH, R. E. H., AND J. KALFF. 1981. The effect of phosphorus limitation on algal growth rates: evidence from alkaline phosphatase. Can. J. Fish. Aquat. Sci. 38: 1421 – 1427.

The inducible enzyme alkaline phosphatase (APA) was used to quantify phosphorus limitation of freshwater phytoplankton growth in situ. Natural phytoplankton communities from oligotrophic–eutrophic Lake Memphremagog were grown at phosphorus-limited rates of 0.06–0.93 per day in continuous, lake water cultures. APA varied inversely with phosphorus-limited growth rate and was little influenced by the community composition changes that occurred in culture. Compared to cultures, average in situ APA in Lake Memphremagog during the ice-free season was low, and suggested phosphorus-limited growth rates greater than 0.6 per day in even the most oligotrophic parts of the lake. Although algal abundance varies with total phosphorus among most north-temperate zone lakes, including Lake Memphremagog, our data show that average community growth rates are normally high and change relatively little over a fourfold range of total phosphorus concentration.

*Key words*: alkaline phosphatase, phytoplankton, growth rate, phosphorus

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La limitation par le phosphore de la croissance du phytoplancton d'eau douce in situ a été quantifiée au moyen de l'enzyme fonctionnante phosphatase alcaline (APA). Des communautés phytoplanctoniques naturelles provenant du lac oligotrophe–eutrophe Memphremagog ont été cultivées à des taux limités par le phosphore de 0,06 et 0,93 par jour dans des cultures continues dans de l'eau du lac. L'APA varie en fonction inverse du taux de croissance limité par le phosphore, et est peu influencée par les changements de composition de la communauté dans la culture. Comparée à celle des cultures, l'APA moyenne in situ dans le lac Memphremagog pendant la saison où le lac est libre de glace est faible et laisse supposer qu'il y aurait des taux de croissance limités par le phosphore qui dépassent 0,6 par jour même dans les parties les plus oligotrophes du lac. Bien que l'abondance des algues varie en fonction du phosphore total dans la plupart des lacs de la zone tempérée septentrionale, y compris le lac Memphremagog, nos données indiquent que les taux de croissance moyens de la communauté sont normalement élevés et changent relativement peu dans une gamme de quatre ordres de grandeur de concentrations de phosphore total.

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DESPITE strong evidence that phosphorus limits phytoplankton abundance and production in many lakes (Schindler 1974), the effect of phosphorus limitation on algal growth rates is difficult to quantify. The relative importance of phosphorus to phytoplankton dynamics, and the implications of changing phosphorus supply to growth and community composition, are consequently poorly understood (Peterson et al. 1974). Measuring growth rates of natural populations is problematic; the most direct method, specific production, is subject to many errors which are especially acute under variable nutrient limitation (Healey 1979). Alternatively, the importance of phosphorus in limiting growth rates can be estimated indirectly by physiological indicators of phosphorus deficiency (Healey and Hendzel 1979). While promising, recent efforts to quantify such indicators in terms of growth rate have yielded only semiquantitative results. The object of the present study is to improve the quantification of one deficiency indicator, alkaline phosphatase, and to report the results of its application in nature.

Alkaline phosphatase (APA) is induced by phosphorus limitation in many phytoplankton species (Kuenzler and Perras 1965; Healey and Hendzel 1975), and is quantitatively related to phosphorus-limited growth rate of some (Fuhs et al.

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1972; Rhee 1973). Sufficient similarity in APA production was found among a group of freshwater phytoplankton species to define APA values characteristic of severe, mild, and no limitation (Healey and Hendzel 1979). In nature, APA correlates with other signs of phosphorus shortage in a number of lakes (Sproule and Kalff 1978; Healey and Hendzel 1980).

To test further the quantitative behavior of APA, we observed its development in continuous-flow cultures of natural phytoplankton communities. The cultures operated on the principle that the cultured populations, after a period of equilibration, come to grow at a rate just balancing the dilution (specific flushing) rate of the phosphorus-limited cultures. Previous use of such cultures (Peterson et al. 1974) suggests that natural populations can be quickly brought to equilibrium, before large changes in community structure occur. The rate and nature of succession, in relation to APA dynamics, were monitored to check this assumption.

The results of the culture experiments were compared to APA observed over a 4-yr period in oligotrophic–eutrophic Lake Memphremagog (Que.–Vt.) to quantify limitation of growth in situ. The lake displays an axial gradient of phosphorus concentration (Carlson et al. 1979) and an accompanying gradient of trophic status (Dermott et al. 1977; Gascon and Leggett 1977; Nakashima and Leggett 1975; Ross and Kalff 1975). Phosphorus deficiency indicators, including APA and orthophosphate concentrations, show that limitation becomes more severe with decreasing total phosphorus along the gradient (Peters 1979; Sproule and Kalff 1978). The APA record thus provides a measure of the influence of phosphorus supply on limitation over a relatively long period.

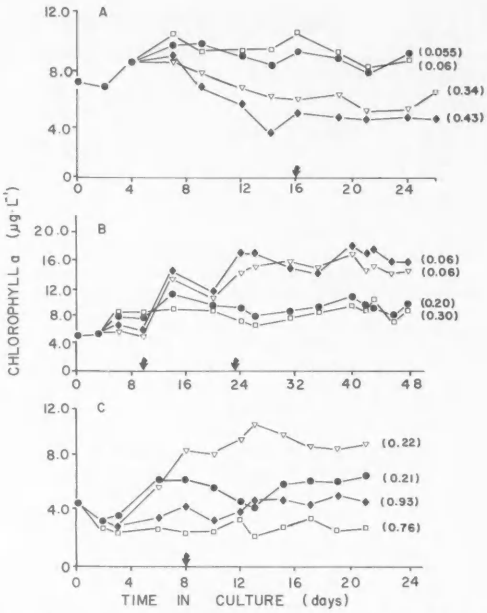


FIG. 1. Representative chlorophyll time courses for experiment 1 (A), 2 (B), and 3 (C); numbers in parentheses are dilution rates (per day) and the arrows mark the onset of APA equilibration; double arrows for experiment 2 are explained in text. Flow started on day 5 (A), 4 (B), and 2 (C).

## Materials and Methods

In each experiment, five or six parallel cultures operated at different dilution rates to produce phytoplankton communities growing at different, phosphorus-limited rates. Chlorophyll and APA were monitored to determine the equilibrium response level. Community and species-specific biomass were routinely monitored for comparison with chlorophyll and APA dynamics. Experiments were performed in July and September, 1978 and October, 1979 (experiments 1, 2, and 3).

The cultures comprised a 1- or 2-L reactor containing the cultured populations, a reservoir of sterile medium, and a pump with silicon tubing manifold to deliver medium to the reactor. The reactors were continuously mixed and bubbled, and were illuminated by cool-white fluorescent lights on a 16:8 LD cycle. The culture-surface light intensity was 200–300 $\mu Ei \cdot m^{-2} \cdot s^{-1}$. The medium was filter-sterilized (0.1-μm membrane filter) lake water enriched with 10% strength, phosphorus-free, synthetic medium (Morgan and Kalff 1979) to ensure phosphorus limitation. Phosphorus was added as $K_2HPO_4$ to match the concentration in the innoculum.

For each experiment, a 10-m integrated sample from central Lake Memphremagog, screened (120-μm mesh) to remove the larger zooplankton, furnished the innoculum. The innoculum also received a 10% phosphorus-free medium enrichment, and a small (2.6 $\mu g\ P \cdot L^{-1}$) phosphorus enrichment to stimulate growth. Pumping started after 2–5 d of acclimation to culture conditions, and dilution rates were calculated by dividing the known flow rate by the reactor volume. Samples for chlorophyll, APA, total phosphorus, and cell counts were taken at 2- to 3-d intervals, keeping sample volume below 10% of reactor volume to minimize perturbation.

Chlorophyll, APA, and total phosphorus were determined at weekly or biweekly intervals in samples from 2 m in depth at four different sites in Lake Memphremagog. The sites were located along the lake's axis to span the nutrient gradient (Sproule and Kalff 1978). Sampling covered at least the June–September period in 1975-77, and July–December in 1974. Data for 1974–75 have been reported previously (Sproule and Kalff 1978).

Chlorophyll *a* was analyzed fluorometrically (Strickland and Parsons 1972) with correction for phaeopigments. Total phosphorus was measured by the molybdenum blue technique following persulfate digestion (Johnson 1971). The APA assay (Hill et al. 1968) used fluorometry and the substrate 3-*O*-methylfluorescein phosphate. Samples (4.5 mL) were mixed with 2.0-μg methylfluorescein (MF)-$PO_4$ in 0.5 mL of pH 8.7 Tris buffer. Change in fluorescence was subsequently monitored using a Turner model 111 fluorometer equipped for high sensitivity (red-sensitive photomultiplier, R-136; high sensitivity door; high intensity blue lamp, F4T5-B) with a

TABLE 1. Ratios of chorophyll (Chl *a*) and biomass (B) to total phosphorus (TP), and ratio of chlorophyll to biomass, averaged over the equilibrium region for cultures of different dilution rates.

| Dilution rate ($d^{-1}$) | Chl *a*:TP (μg:μg) | B:TP (mg:μg) | CHLa:B (μg:mg) |
|---|---|---|---|
| | *Experiment 1* | | |
| 0.055 | 0.75 | 0.25 | 3.0 |
| 0.060 | 0.76 | 0.22 | 3.4 |
| 0.22 | 0.66 | 0.17 | 3.9 |
| 0.34 | 0.57 | 0.16 | 3.5 |
| 0.43 | 0.51 | 0.10 | 5.1 |
| | *Experiment 2* | | |
| 0.06 | 0.61 | 0.46 | 1.3 |
| 0.06 | 0.65 | 0.44 | 1.5 |
| 0.20 | 0.82 | 0.43 | 1.9 |
| 0.30 | 0.91 | 0.44 | 2.1 |
| 0.43 | 0.88 | 0.34 | 2.6 |
| | *Experiment 3* | | |
| 0.21 | 0.27 | 0.13 | 2.1 |
| 0.22 | 0.50 | 0.49 | 1.0 |
| 0.32 | 0.60 | 0.54 | 1.1 |
| 0.34 | 0.52 | 0.55 | 1.0 |
| 0.76 | 0.63 | 0.64 | 1.0 |
| 0.93 | 0.37 | 0.34 | 1.1 |

Wratten 47-B primary filter and a Wratten 2A-12 plus a 10% transmission neutral density filter on the secondary side. Fluorescence was measured at intervals appropriate to the reaction rate (1−10 min) within the period of linear response (change of 100 fluorescence units or less). The reaction rate was expressed as μg methylfluorescein per millilitre per hour. Both soluble (passing a washed 0.45-μm membrane filter) and total activity were measured at room temperature. The particulate activity, found by difference and normalized to chlorophyll, is the fraction considered here.

Phytoplankton, preserved with Lugol's iodine, was counted by the Utermöhl method on an inverted, phase contrast microscope. Three hundred or more cells in total were counted for each sample. Species and total biomass were estimated from cell volumes calculated by simple geometric approximations, using dimensions measured on at least 25 individuals for common species and assuming a specific gravity of unity.

## Results

Chlorophyll and APA dynamics were followed in each experiment to identify the equilibrium response. All experiments showed an initial lag phase, with chlorophyll decreasing slightly or remaining constant (Fig. 1). Subsequently, chlorophyll usually increased directly to a plateau, or equilibrium value, which varied among cultures (Fig. 1B, C). In some cultures of experiment 1, chlorophyll first increased after lag phase and subsequently decreased to a plateau value (Fig. 1A). Experiment 2 (Fig. 1B) showed that equilibria persisted with little systematic change for up to 48 d.

Equilibrium chlorophyll concentration varied directly with total phosphorus concentration in the medium (correlation coefficient = 0.83, $P < 0.01$). The atypical chlorophyll dynamics seen in experiment 1 (Fig. 1A) corresponded to cultures which received medium of (accidentally) lower phosphorus concentration than that in the reactor at the outset. Chlorophyll concentrations increased in response to enrichment of the reactor, and subsequently decreased as reactor contents equilibrated with the medium.

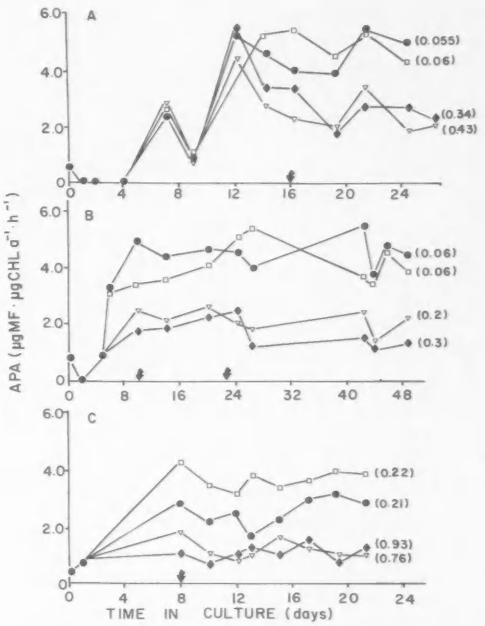


FIG. 2. Representative time courses of APA for experiments 1−3 (A−C), corresponding to the chlorophyll time courses (Fig. 1).

The ratio of chlorophyll to total phosphorus did not vary consistently with dilution rate (Table 1). The ratio varied inversely with dilution rate in experiment 1, but showed a slight opposite trend in experiment 2 and no trend in experiment 3. This was not simply the result of change in the ratio of chlorophyll to biomass (community cell volume) (although the ratio did increase with dilution rate in experiments 1 and 2) because the ratio of biomass itself to total phosphorus changed little with dilution rate in experiment 2 and not at all in experiment 3 (Table 1). Like chlorophyll, however, total biomass increased significantly with total phosphorus among cultures ($r = 0.88$, $P < 0.01$), and was also correlated with chlorophyll ($r = 0.60$, $P < 0.05$).

Dynamics of APA (Fig. 2) also showed equilibration in each culture. Dynamics of APA resembled chlorophyll, usually increasing quite directly to equilibrium (Fig. 2B, C), but paralleling the delayed approach to equilibrium of chlorophyll in some cultures (Fig. 2A). The relatively long-term experiment 2 again suggested that equilibria were quite stable, if noisy, over a period of several weeks at least (Fig. 2B).

Equilibrium APA decreased with increasing dilution rate in each experiment (Fig. 2). The pooled results showed an in-

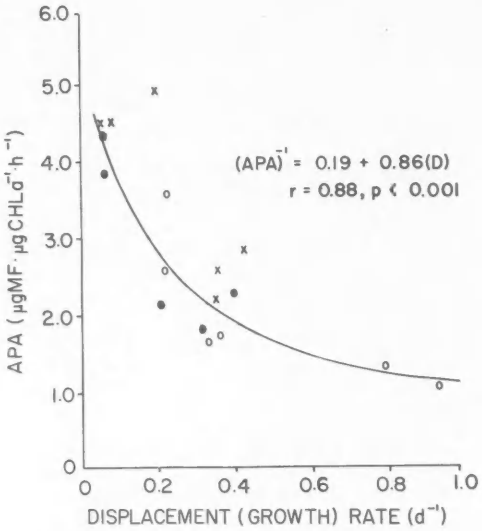


FIG. 3. Equilibrium APA versus dilution rate for experiment 1 (crosses), 2 (closed circles), and 3 (open circles). Curve and equation represent the line fitted by linear regression.

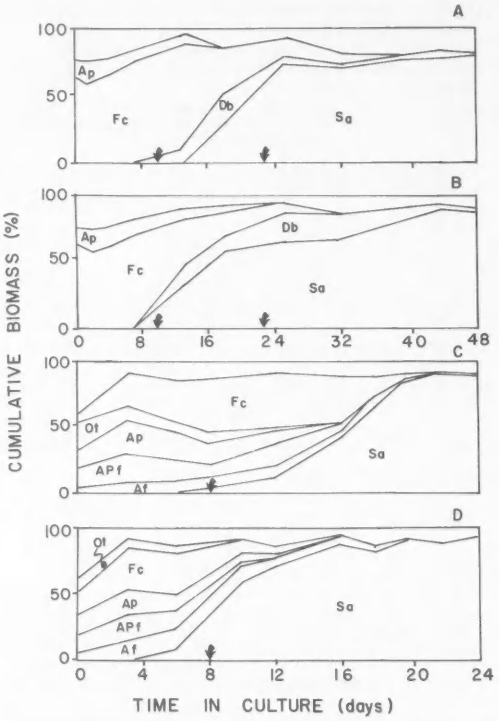


FIG. 4. Representative cumulative biomass distributions for experiment 2 (A, B) and 3 (C, D). Highest (B, D) and lowest (A, C) dilution rate cultures (Fig. 2) are shown for each experiment. The arrows denote equilibration of APA. Symbols are Sa, *Synedra acus*; Db, *Dinobryon bavaricum*; Fc, *Fragilaria crotonensis*; Ap, *Anabaena planctonica*; APf, *Aphanizomenon flos-aquae*; Af, *Asterionella formosa*; Ot, *Oscillatoria tenuis*.

verse, nonlinear relationship between equilibrium APA and dilution, or growth rate (Fig. 3), with steepest APA response at low growth rates. APA ranged from 4.9 to 1.0 μg MF · μg Chl $a^{-1} \cdot h^{-1}$ over the range of growth rates from 0.055 to 0.93 per day.

Species composition changed with time in all cultures. In experiments 2 and 3, all cultures became dominated by the diatom *Synedra acus* (Fig. 4). Both experiments were initially dominated by diatom and blue-green algae, with diatoms relatively more important in experiment 2 than in experiment 3. The greatest change in composition occurred between days 8 and 20.

Succession was slower and qualitatively different in experiment 1 (Fig. 5). Of six cultures in the experiment, one was dominated by *S. acus*, another by the chrysophycean *Dinobryon bavaricum*, and four had three or more species co-dominant throughout. Blue green algae were not important in the initial or final biomass of the community in experiment 1.

The onset of equilibrium in APA appeared to precede major shifts in species composition (Fig. 2, 4, 5). The equilibrium values of APA in experiment 2 were calculated both from plateau values obtaining before and during the shift to *S. acus*, and from values observed after dominance was complete (Fig. 2B, 4A, B). A nonparametric test (Mann–Whitney; Connover 1971) revealed no significant difference between the two estimates in any of the five cultures in the experiment. Equilibrium APA (Fig. 3) was thereafter calculated from the entire equilibrium region.

The APA record from Lake Memphremagog comprises 4 years of observations at four stations along the lake's axis (Fig. 6). Generally APA was less than unity in the lake, even at the most oligotrophic site, but there were periods of relatively high APA. In the 2 years with May samples, a large spring peak of 2.0 μg MF · μg Chl $a^{-1} \cdot h^{-1}$ occurred in most basins. The peaks quickly subsided to lower values, which generally persisted with considerable oscillation throughout the summer (June–Sept.). In the more eutrophic sites APA declined yet further into the autumn. A late summer or autumn peak occurred at the oligotrophic stations in 1975, decreasing sharply later in the autumn. The average value of APA over the summer (June–Sept.) varied inversely with average total phosphorus over the same period (Fig. 7). Despite the large seasonal variation of APA, and changes in the phosphorus concentrations between years, the relationship explained 66% of the APA variation. In summer, average APA ranged from about 0.4 to 1.5 μg MF · μg Chl $a^{-1} \cdot h^{-1}$ and total phosphorus from 30 to 8 μg $P \cdot L^{-1}$ in the lake (Fig. 7).

## Discussion

A drawback of continuous flow cultures in manipulating community nutrient status is the trade-off between equi-

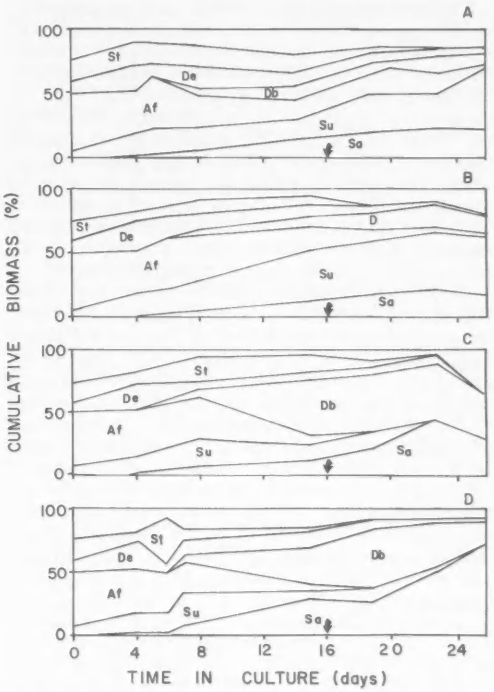


FIG. 5. Representative cumulative biomass distributions for the four cultures of experiment 1 shown in Fig. 1A and 2A, from lowest dilution rate (A) to highest (D). Symbols are Su, *Synedra ulna*; De, *Diatoma vulgare* var. *elongatum*; St, *Stephanodiscus* sp.; others as Fig. 4.

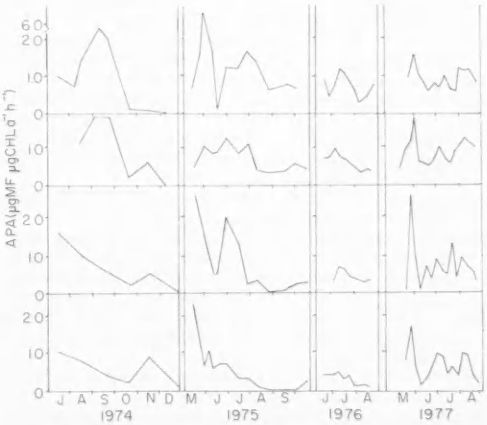


FIG. 6. APA dynamics in Lake Memphremagog. The bottom panel represents the most southerly and eutrophic site, and the top panel the most northerly and oligotrophic site.

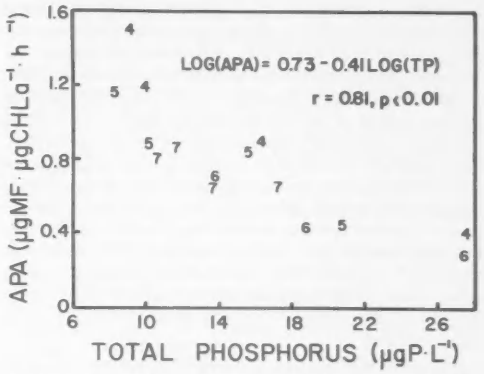


FIG. 7. Summer (June–Sept.) average APA versus total phosphorus in Lake Memphremagog. The equation describes the line fitted by linear regression. The symbols are different years of observation (1974–77) and each point is an average for one site (Fig. 6).

libration with the nutrient supply and change over time of community composition from the natural. In our cultures succession greatly simplified species composition, leading to diatom-dominated communities with, very often, a large preponderance of *S. acus* (Fig. 4). However, the equilibration of APA did not depend on dominance of *S. acus* because APA values before and after establishment of dominance in experiment 2 were the same, and in the other experiments APA equilibrated earlier than species composition. The relatively diverse cultures of experiment 1 yielded APA somewhat higher than the average relationship of Fig. 3, but there was no apparent relationship between the magnitude of residuals and species composition; the especially large outliers included mixed and *S. acus*-dominated cultures, while two mixed cultures gave excellent agreement with the *S. acus*-dominated cultures of other experiments. All these observations show that the range of species composition encountered in the cultures from Lake Memphremagog is not of great significance to APA response.

Equilibration of community growth with the nutrient supply appears more important than species composition to APA response. Changing nutrient supply delayed equilibration of population size and APA (Fig. 1A, 2A) and likely contributed to the relatively high APA values of experiment 1 (Fig. 3). The sensitivity of APA to equilibration makes difficult the interpretation of APA data from batch monocultures, which comprises most of the data on interspecific differences in APA production (Kuenzler and Perras 1965; Healey and Hendzel 1979). Some interspecific differences in APA response reported probably reflect failure of the batch cultures to equilibrate before onset of senescence, a common problem in batch studies of physiological processes (Fogg 1975).

Total biomass at equilibrium is frequently a decreasing function of dilution rate in phosphorus-limited monocultures (Rhee 1973; Healey and Hendzel 1975; Goldman 1977) but neither chlorophyll nor biomass (cell volume), once corrected for differences of total phosphorus concentration among cultures, was consistently related to dilution rate in our mixed species culture experiments (Table 1). Much of the apparent

variability of chlorophyll and biomass to total phosphorus ratios among experiments was probably caused by a variable concentration in the medium of soluble, but largely unavailable, phosphorus present in Lake Memphremagog water (Peters 1979). Further, however, any biomass unit other than cellular content of the limiting nutrient itself is difficult to interpret because such measures vary with both nutrient deficiency and, to some extent, taxonomy. Our variable composition ratios (Table 1) illustrate this for some mixed cultures and show that neither chlorophyll nor community volume is an ideal biomass measure. Our results with APA might therefore have been stronger with a better biomass measure than chlorophyll, but chlorophyll does have the advantage of easy measurement on field samples and a demonstrable, if imperfect, correlation with both total phosphorus supply and community volume.

Equilibrium APA varied inversely with dilution rate, indicating that the enzyme is quantitatively related to community growth rate despite underlying changes in species composition. The nonlinear response (Fig. 3) was similar to some reported from monoculture (Fuhs et al. 1972; Rhee 1973), although other forms have been reported (Healey and Hendzel 1975). Response form (Fig. 3) was heavily influenced by two points at high dilution rate, and better definition of the response curve is an important goal for future work. The curve (Fig. 3) is the first such function for natural populations, and provides a first quantification of limitation in situ.

The highest summer-average APA values observed in situ (Fig. 7), representing the most oligotrophic locations in the lake, corresponded to phosphorus-limited growth rates of 0.6 per day or more (see Fig. 3). The lowest APA values in Fig. 7 indicated, by extrapolation from Fig. 3, growth rates greater than 2.0 per day. By comparison, many phytoplankton species have growth rates, under optimal culture conditions, of 1.0–2.0 per day at 18–22°C (Fogg 1975; Eppley and Sloan 1966; Williams 1964). Average growth rates as large as 2.0 per day in situ seem unlikely, however. Despite problems in carbon-14 methodology (Peterson 1980), its application to many lakes indicates that sustained growth rates much greater than 1.0 per day are rare even in the optimal light layer of nutrient-rich lakes (Ahlgren 1970). The APA observed in Lake Memphremagog therefore may well reflect near phosphorus sufficiency in eutrophic sites, and limitation at oligotrophic sites of at least half of typical nutrient-saturated rates.

Community average growth rates encompass a range of species-specific growth rates, and individual species may not follow the pattern described here. In fact, the species initially dominant in the lake were excluded during the cultures' equilibrium phase, and were accordingly growing more slowly than the dilution rate, while flagellate and other nannoplankton never grew well in our cultures. This would bias our interpretation of community growth rates in situ if the succeeding species, which were able to grow faster than the dilution rate (otherwise community biomass would decline), were characteristically different in APA production from their predecessors. Evidence that this is not the case has already been mentioned. Instead, the APA production in culture appears to be the average contributed by species growing at rates both above and below the dilution rate, but having a similar APA response.

Confirming the indication from APA that high growth rates can be supported throughout much of Lake Memphremagog, orthophosphate concentrations also show a gradient of mild limitation. Peters (1979) used radiobioassay to measure summer average orthophosphate concentrations of 1.7, 1.2, and 0.6 $\mu g\ P \cdot L^{-1}$ from eutrophic to oligotrophic sites in 1976. By comparison, the seven half-saturation growth constants noted in a quick survey of the nutrient kinetic literature showed five below 1.0 $\mu g\ P \cdot L^{-1}$, and only one above 2.0 (Tilman and Kilham 1976; Kilham et al. 1977; Fuhs et al. 1972; Rhee 1973; Burmaster 1979). Like APA, this indicates roughly half-maximal growth at the most oligotrophic site, and even less severe limitation elsewhere in the lake.

Growth rate limitation in Lake Memphremagog appears typically mild despite evidence that phosphorus limits phytoplankton abundance and production (Ross and Kalff 1975; Sproule and Kalff 1978; Carlson et al. 1979). Seasonal dynamics (Fig. 6) reveal only brief episodes of APA high enough to reduce growth rates much below 0.5/d. A number of other phosphorus-limited lakes have also shown little sign of severe growth rate reduction (Peterson et al. 1974; Healey and Hendzel 1980). Our data suggest that over the range of 6–28 $\mu g\ P \cdot L^{-1}$, a range encompassing oligotrophic and meso–eutrophic lakes, average summer growth rates of 0.6/d or greater can be supported (Fig. 7, 3). Thus, even though algal abundance decreases with decreasing total phosphorus over this range (Kalff and Knoechel 1978), growth rates continue to be high.

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# The Structure of Crustacean Zooplankton Communities in Glaciated Eastern North America

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The zooplankton community structure of 696 lakes in Pleistocene glaciated eastern North America indicated to us that glacial influence was the primary determinant of community structure. Using this criterion we successfully discriminated 82% of the lakes between glacial lakes, to which *Diaptomus sicilis*, *Limnocalanus macrurus*, *Mysis relicta*, and *Senecella calanoides* were restricted, and nonglacial lakes, to which *Cyclops scutifer*, *Eubosmina longispina*, *Daphnia pulex*, *D. catawba*, and other species were characteristic. A third large group of common species was distributed throughout the study area with no particular relation to glacial lake influence. No clear patterns in community structure with respect to size of zooplankters were discerned. Although some species distributions or abundances were consistent with the well-known predatory and competitive interactions among zooplankters typical of small lakes and ponds, for example, the negative correlation between the predatory *Mysis relicta* and *Eubosmina longispina*, most were not. We suggest that this arose partly because the study area is large and heterogeneous and partly because the biological interactions observed in small lakes and ponds may be less intense in the more physically complex environment of large lakes.

*Key words*: zooplankton, community structure, glacial opportunists, multivariate analysis, zoogeography, size-efficiency hypothesis

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La structure des communautés zooplanctoniques de 696 lacs de la partie de l'est de l'Amérique du Nord touchée par la glaciation du Pléistocène est pour nous une indication que l'influence glaciaire est le facteur déterminant de la structure des communautés. Ce critère nous a permis de différencier correctement 82% des lacs : lacs glaciaires, dans lesquels *Diaptomus sicilis, Limnocalanus macrurus*, *Mysis relicta* et *Senecella calanoides* sont restreints, et lacs non glaciaires caractérisés par *Cyclops scutifer*, *Eubosmina longispina*, *Daphnia pulex*, *D. catawba* et autres espèces. Un troisième groupe important d'espèces communes est réparti dans toute la région d'étude, sans qu'il y ait relation particulière avec l'influence de lacs glaciaires. Nous n'avons pu détecter de patron particulier dans la structure des communautés quant à la taille des zooplanctontes. La répartition et l'abondance de certaines espèces s'accorde avec les interactions prédatrices et compétitives bien connues chez les zooplanctontes typiques de petits lacs et étangs. C'est le cas, par exemple, de la corrélation négative entre *Mysis relicta* et *Eubosmina longispina* prédateurs. Cependant, cette concor-

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dance est l'exception plutôt que la règle. Ceci serait dû en partie à l'étendue et à l'hétérogénéité de la région étudiée, et en partie à des interrelations biologiques observées dans des petits lacs et étangs qui seraient moins intenses dans l'environnement plus complexe des grands lacs.

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RECENT North American studies of the relation between the structure of freshwater zooplankton communities and physicochemical features of lakes have been made in restricted geographical areas of rather homogeneous character (Patalas 1971; Pinel-Alloul et al. 1979; Roff and Kwiatkowski 1977; Sprules 1977). From these studies it is unclear whether zooplankton species associations typical of a given morphoedaphic lake type tend to be the same among regions as one would expect given the widespread distributions of component species (Carter et al. 1980) or species associations tend to vary among regions as one would expect if local geologic history and patterns of species invasions are more important than biological interactions. If species associations do vary among regions, the frequency distribution of body sizes of zooplankton (Sprules and Holtby 1979) could nevertheless be maintained; this would be expected on the basis of the well-known (at least for ponds and small lakes) size-related effects of competition and predation on zooplankton (Brooks and Dodson 1965; Hall et al. 1976; Kerfoot 1978, 1980; Lynch 1979).

None of these expectations could previously be evaluated because community analyses of freshwater zooplankton over large, heterogeneous geologic areas had not been attempted. The recent study of Carter et al. (1980) has provided data on zooplankton species in a large sample of lakes stretching over most of eastern North America; however, that study was essentially limited to considerations of individual species distributions and did not consider the biological environment or species interactions.

In the present study we investigated the patterns of zooplankton community structure in the lakes of Carter et al. (1980) to determine what species coexist and in what combinations, and we examined some of the possible reasons underlying the observed patterns of coexistence. We show that glacial influence is the major correlate of zooplankton community structure but that associated species patterns are generally weaker than those in more restricted areas. We interpret the lack of clear patterns in frequency distribution of body sizes in zooplankton to indicate that effects of size-selective predation and competition may be less intense in the physically more complex environment of large lakes.

## Material and Methods

The whole data set of Carter et al. (1980) was availabe for the analyses presented here. This set comprised coded abundances of numbers per unit surface area (0 = not observed; 1 = rare (≤10); 2 = common (11−250); 3 = abundant (>250)) of 45 planktonic crustaceans and four dipteran species in 696 lakes of glaciated eastern North American sampled during the summers of 1969 to 1978. These codes effectively represent a logarithmic transformation of the raw species abundances. Adults of all calanoid and cyclopoid species and all identifiable sizes of cladocerans and dipterans were enumerated. Presence−absence data for six fish species as well as limnological data were also available. Other collection methodology, analytical procedures, and identification techniques for these lakes have been presented by Carter et al. (1980). The area studied covers Canada and the United States between approximately 41 and 53°N and 84°W and the Atlantic Ocean. The three physiographic regions represented are the eastern Canadian Shield, the St. Lawrence Lowland, and northern Appalachia (see Carter et al. 1980).

To reduce gaps in the data set to a minimum, the whole data matrix was first reduced to 522 lakes by eliminating 174 lakes with incomplete species and physicochemical data; the lakes remaining were essentially those of Dadswell (1974) sampled with a 239-μm-mesh net (see Carter et al. 1980). Of the 49 plankton species, 22 occurring in fewer than 10% of the lakes (a natural hiatus in the frequency distribution of species in lakes) were removed, and only physicochemical variables missing values in fewer than 15% of the lakes (again a natural break in the data) were retained. In addition to standard limnological variables, a modified Ryder's index (Ryder et al. 1974; see Table 2), the only production-related variable which could usefully be extracted from the data, was used. Lakes were also classified according to glacial lake influence (Carter et al. 1980; Dadswell 1974), by which we mean lakes whose basins were once part of the large interconnected Great Glacial Lake systems of central North America. These modifications produced a data matrix of 15 physicochemical variables and categories of abundances of 27 species in 522 lakes.

Patterns of community structure in the lakes were summarized by a principal components analysis (PCA) of the species variance−covariance matrix. Resulting species patterns were related to the limnological characteristics by computing Spearman rank correlations between the scores of lakes on a principal component and the physicochemical data.

Because the efficiency of PCA as used above may be considerably reduced by nonlinear species responses to environmental gradients (Gauch and Whittaker 1972; Austin and Noy-Meir 1971), a subset of plots of the transformed species abundances against physicochemical variables was examined. Despite the scatter in these relations the trends were often linear, or at least monotonic, but never the bell-shaped distributions considered by Austin and Noy-Meir (1971) to affect so drastically ordination techniques. PCA is thus a suitable statistical model for the analysis presented here. Other statistical models such as cluster analysis of mean Euclidean distances among lakes, reciprocal averaging and data transformations such as proportionate species abundances and its arc sin square root (Sprules 1977) did not improve upon a PCA of coded species abundances for pattern identification. Only recurrent group analysis (Fager and McGowan 1963; Sprules

1975), a clustering procedure based on species presence–absence data, proved a useful adjunct to PCA and is used here for corroboration of the community patterns.

For comparison with previous studies in which patterns of cogeneric occurrences were considered a measure of interspecific competition the coexistence of species within the families Bosminidae and Cyclopodiae and the genera *Daphnia* and *Diaptomus* are presented. Frequencies of individual species in 666 lakes were determined and the actual number of lakes containing a particular species group was compared with that expected under the null hypothesis of random assortment. The additional lakes examined comprised most of those sampled by Carter (see Carter et al. 1980) with a 158-μm-mesh net.

Because size structure in zooplankton communities is more likely to be maintained than is taxonomic structure over broad geographic regions (Sprules and Holtby 1979; Sprules 1980), a second "ecological" components analysis of these data was performed by first grouping the original 49 species into six size categories on the basis of adult body length as reported in Edmondson (1959). The rationale and processes involved in this approach are explained in Sprules and Holtby (1979). The abundance of a particular size category in a given lake was taken as the coded geometric mean of its component taxonomic species. This left a matrix of coded abundances of six size categories in the 522 lakes. Principal components were extracted from the size category variance–covariance matrix and were correlated with limnological characteristics of lakes as before.

Preliminary statistical analyses were done at the University of Guelph Computer Centre using ORDIFLEX (Gauch 1977), a flexible program for various ordination techniques. Final analyses were run at the University of Toronto Computer Centre using GENSTAT (Alvey et al. 1977), a package of powerful matrix and statistical operations, SPSS (Nie et al. 1975), and some simple FORTRAN procedures. Initial recurrent group analyses were run at the University of Toronto and confirmed at the University of Guelph using independently written FORTRAN programs.

## Results and Discussion

### Multispecies Associations

The first three principal components from analysis of the distribution of 27 zooplankton species in 522 lakes accounted for a total of 34.5% of the variance in the data, the first component accounting for 15.1% of the variance, the second for 12.2%, and the third for the remaining 7.2%. The next three components (four to six) only accounted for a further 16.4% of the variance. Most species were significantly correlated with the components (Table 1) because of the large number of degrees of freedom (520). *Diaphanosoma* spp. (see Carter et al. 1980, *Diacyclops bicuspidatus thomasi*, and *Mysis relicta* have the highest positive correlations with the first component and *Cyclops scutifer* and *Eubosmina longispina* have the highest negative correlations. The primary contrast in community composition is between lakes having relatively high abundances of the first group of species and those having relatively high abundances of the second group.

TABLE 1. Correlations of zooplankton abundances with components 1 through 3 from taxonomic PCA. (Correlations greater than 0.08 are significant at $P \leq 0.05$.)

| | Principal component | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| *Diaphanosoma* spp. | 0.63 | −0.31 | −0.08 |
| *Diacyclops bicuspidatus thomasi* | 0.56 | −0.15 | 0.28 |
| *Mysis relicta* | 0.54 | 0.56 | −0.08 |
| *Daphnia galeata mendotae* | 0.49 | −0.31 | 0.05 |
| *Diaptomus oregonensis* | 0.45 | −0.46 | 0.49 |
| *Daphnia retrocurva* | 0.44 | −0.14 | −0.01 |
| *Senecella calanoides* | 0.40 | 0.23 | −0.25 |
| *Diaptomus sicilis* | 0.35 | 0.51 | −0.07 |
| *Epischura lacustris* | 0.35 | −0.37 | −0.35 |
| *Mesocyclops edax* | 0.34 | −0.34 | 0.0 |
| *Leptodora kindtii* | 0.34 | −0.40 | −0.24 |
| *Limnocalanus macrurus* | 0.33 | 0.37 | −0.11 |
| *Bosmina longirostris* | 0.28 | 0.25 | −0.10 |
| *Acanthocyclops vernalis* | 0.22 | −0.04 | 0.19 |
| *Ceriodaphnia lacustris* | 0.20 | −0.03 | 0.17 |
| *Polyphemus pediculus* | 0.19 | −0.12 | −0.20 |
| *Chaoborus punctipennis* | 0.19 | −0.34 | 0.09 |
| *Daphnia dubia* | 0.16 | −0.37 | −0.03 |
| *Sida crystallina* | 0.13 | −0.31 | −0.18 |
| *Eubosmina tubicen* | 0.06 | −0.14 | −0.14 |
| *Holopedium gibberum* | 0.05 | −0.35 | −0.78 |
| *Diaptomus minutus* | 0.04 | −0.11 | −0.20 |
| *Daphnia catawba* | −0.10 | −0.19 | −0.09 |
| *Daphnia longiremis* | −0.18 | −0.54 | 0.06 |
| *Daphnia pulex* | −0.23 | −0.11 | 0.21 |
| *Eubosmina longispina* | −0.38 | −0.46 | −0.15 |
| *Cyclops scutifer* | −0.63 | −0.26 | 0.13 |

The strongest limnological correlate of the first component is glacial lake influence (Table 2), suggesting that connection to the Great Glacial Lakes system was an important determinant of the primary pattern in zooplankton community structure. A secondary pattern in species variation contrasts lakes having comparatively high abundances of *M. relicta*, *Diaptomus sicilis*, *Limnocalanus macrurus*, *Bosmina longirostris*, and *Senecella calanoides* with those having relatively high abundances of *Daphnia longiremis*, *Diaptomus oregonensis*, and *E. longispina* (Table 1, PC 2). Again glacial lake influence is strongly related to this pattern in community structure (Table 2). Although there is some overlap, this primary division of lakes and their zooplankton communities by geological history is striking (Fig. 1). A discriminant function analysis showed that it was possible, by simply using lake scores on PC 1 and PC 2, to assign correctly 82% of the lakes to their appropriate category, i.e. glacially influenced or not.

The nature of the relation between lake influence and community structure is supported by further analysis of the data. Recurrent group analysis (RGA) of the 522 lake set (Fig. 2) identifies three species groups, two with associated outliers. *Cyclops scutifer* and *E. longispina*, which comprise the smallest group, load heavily on the negative end of PC 1 (Table 1) and are disproportionately frequent in lakes situated outside former glacial lake boundaries (here termed nonglacial lakes) (Table 3). Hence these species and others showing similar negative values in Tables 1 and 3 tend to be characteristic of nonglacial lakes. *Bosmina longirostris*, *Diaphanosoma* spp.,

TABLE 2. Spearman rank correlations of taxonomic components with limnological variables. (Values greater than 0.09 are significant at $P \leq 0.05$.)

| | Principal component | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Influence[a] | 0.52 | 0.39 | 0.01 |
| Modified Ryder's index[b] | 0.32 | 0.09 | 0.36 |
| Epilimnion pH | 0.28 | 0.18 | 0.39 |
| Total hardness (mg/L) | 0.23 | 0.22 | 0.47 |
| Basin area ($km^2$) | 0.23 | 0.02 | −0.12 |
| Color (mg/L) | 0.19 | −0.07 | −0.08 |
| Max. hypolimnetic temp. (°C) | 0.17 | −0.09 | −0.21 |
| Surface area (ha) | 0.16 | 0.07 | −0.22 |
| Depth (m) below which $[O_2] < 2$ mg/L[c] | 0.13 | 0.00 | 0.30 |
| Hypolimnion pH | 0.10 | 0.27 | 0.34 |
| Lake volume ($m^3$) | 0.08 | 0.11 | −0.20 |
| Maximum depth (m) | −0.07 | 0.11 | 0.04 |
| Elevation (m) | −0.15 | −0.20 | −0.36 |
| Mean depth (m) | −0.19 | 0.16 | 0.04 |
| Secchi depth (m) | −0.24 | −0.01 | −0.06 |

[a] 1 = neither glacial nor marine; 2 = marine only; 3 = glacial only; 4 = both glacial and marine (see Carter et al. 1980; Dadswell 1974).

[b] $\text{Log}_e$ (total hardness/mean depth).

[c] This depth scaled through division by maximum depth.

and *M. relicta* make up a second recurrent group with three associated species; all six species occur disproportionately in lakes occupying former glacial lake basins (here termed glacial lakes) (Table 3) and four of them have high positive correlations with PC 2 (Table 1). These species along with a few others, notably *L. macrurus*, showing positive values in Table 3 thus tend to characterize glacial lakes.

Finally, the largest recurrent group (Fig. 2) comprises nine species and a single associated species *Chaoborus punctipennis*, which by and large show no particular relation to glacial lake influence (Table 3). *Diacyclops bicuspidatus thomasi* and *D. longiremis* have an equivocal position because of intermediate level association with glacial and nonglacial lakes, respectively. In general this large group comprises widely distributed, common species which are characteristic of other Precambrian lake systems (Sprules 1975, 1977). Because nonglacial species load negatively on PC 1 and a mixture of glacial and wide ranging species load positively, this component primarily contrasts nonglacial lakes with all others. Similarly, PC 2 with positive loadings of glacial species and negative loadings of all others contrasts glacial lakes with other lakes. PC 3 accounts for very little of the total community structure variation but it is worth noting that *Holopedium gibberum* has a high negative correlation with this component which in turn is positively related to water hardness (Tables 1 and 2). This corroborates the observation of Carter et al. (1980) that *H. gibberum* occurs more frequently in soft waters.

The first three components from the "ecological" analysis accounted respectively for 38.0, 18.9, and 15.1% of the variation in size structure for a total of 72.0%. With the possible exception of the second component, which contrasted the largest and smallest size-classes with intermediate ones, there were no clear patterns in this analysis (Table 4). There

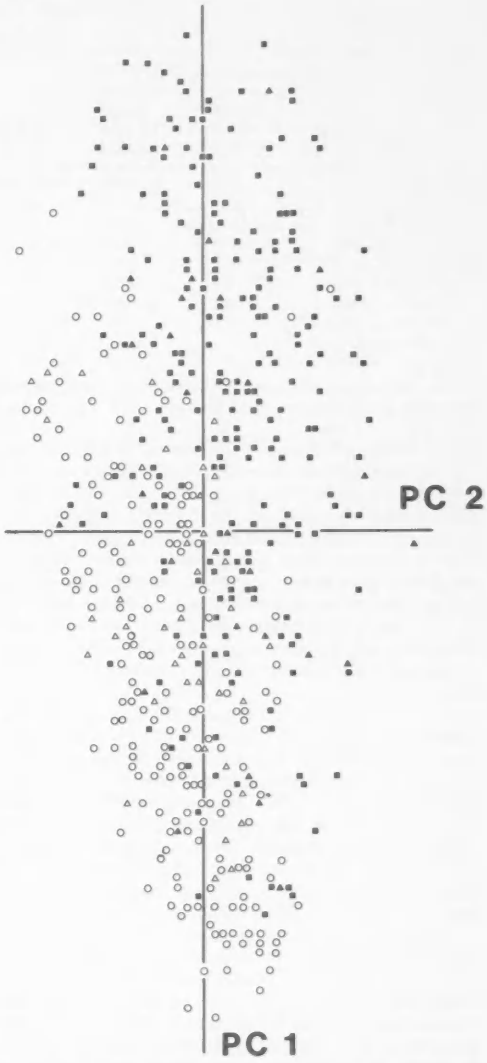


FIG. 1. Principal component ordination from taxonomic study of 522 lakes. ■, Glacial-influenced lakes (not marine influenced); △, marine-influenced lakes (not glacially influenced); ▲, glacial- and marine-influenced lakes; ○, lakes not glacially or marine influenced. PC 1, 2 = principal components 1 and 2.

appeared in most cases to be a mixture of all major size groups with comparatively low correlations between the components and the limnological variables (Table 5).

A number of important points emerge when comparing these community patterns to those from specific regions of Ontario. The first three principal components only accounted for 34.5% of the species variance in this study whereas in a

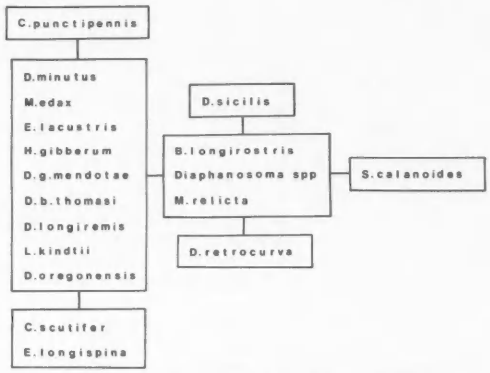


FIG. 2. Recurrent groups and their interrelationships in 522 lakes in Pleistocene glaciated eastern North America.

similar analysis of lakes in the Haliburton, Bruce Peninsula, and Experimental Lakes areas (Sprules 1971) they accounted for 67.8, 55.8 and 60.7% of the variance, respectively. Thus, although there are clearly recognizable trends in species associations over a heterogeneous geographic area, they tend not to be as strong as those within smaller regions which are presumably more homogeneous with respect to geography, geology, and the zoogeographic history of the species. From one region to next the patterns are different, so that by combining them as in the analysis presented here for an extensive region, patterns in different areas tend to cancel one another out.

A second important point concerns the glacial–nonglacial dichotomy in these plankton communities. It is well established that species such as *S. calanoides*, *M. relicta*, and *L. macrurus* are glacial opportunists (Dadswell 1974). In addition, Carter et al. (1980) proposed that *D. sicilis* and *Diaptomus ashlandi* be considered glacial opportunists because their distribution is closely related to Pleistocene glacial events. However, some other species (e.g. *B. longirostris*, *Daphnia retrocurva*, and *Diaphanosoma* spp.), which here show association with glacial lakes, have not previously been considered as influenced by glacial history. Hence the possibility arises that the primary glacial opportunists control the success of other species, through biological interactions. It may be, for instance, that *E. longispina* appears disproportionately frequently in nonglacial lakes primarily because it is barred from glacial lakes by the predator *M. relicta*; such a negative interaction between a bosminid and *Mysis* has been observed in Lake Tahoe (Goldman et al. 1979). Similarly, a species such as *Diaphanosoma* spp. may appear restricted to glacial lakes simply because a species it would otherwise interact with has been eliminated from glacial lakes by one of the glacial opportunists. Clearly further clarification of this point would require extensive experimental data.

It appears that the best intepretation of species patterns over the geographic area studied here is based on the recurrent group analysis (Fig. 2). One group of species occurs primarily in glacial lakes either because of easier access during deglaciation (Dadswell 1974) or from secondary biological effects of the "controlling" glacial opportunists. A second group of species occurs primarily in nonglacial lakes possibly because they are unable to coexist with the glacial opportunists. A third group of species is distributed independently of lake origin and presumably also independently of the glacial opportunists.

A third point concerns the ecological or size analysis of the species. In contrast with this same analysis for the Bruce Peninsula lakes (Sprules and Holtby 1979) there is little consistency to the results presented here. No obvious size structure of plankton communities exists over the broad region of eastern North America during the summer months. We had originally hypothesized that lack of pattern in taxonomic structure might be due to species replacement from one region to another but that overall size structure (or ecological roles) would be maintained. This is clearly not the case here in marked contrast with the expectations of the size-efficiency hypothesis (Brooks and Dodson 1965) which predicts distinct community size patterns as a function of the presence of planktivorous fish and invertebrate predators. In small experimental ponds (Hall et al. 1970) and small natural ponds in ecologically extreme areas (Sprules 1972; O'Brien and Schmidt 1979; Dodson 1975) where biological interactions are presumably intense the expected size patterns occur. However, in the large, deep lakes of glaciated North America where considerable vertical and horizontal environmental heterogeneity presumably ameliorates intense interaction, clear size patterns based on mature animal sizes do not exist.

## NEGATIVE INTERSPECIES CORRELATIONS

The positive associations among individual species have been largely dealt with in RGA (Fig.2). Any discrepancies between the positive correlations (Table 6) and RGA are probably because the latter analysis deals only with presence–absence data whereas the correlation matrix is based on abundance codes. RGA is thus to be preferred as a measure of positive species associations among lakes.

Some comments on the negative correlations between species (according to abundance codes) are warranted (Table 6). We have considered the negative correlations to fall into three groups.

a) Our data set contains both deep and shallow lakes (see Carter et al. 1980). Species characteristically epilimnetic could occur in all lakes whereas hypolimnetic forms can only occur in deep lakes. Hence purely as an artifact of the distribution of lake depths, some species pairs could be negatively correlated. Such probable cases are designated "a" in Table 6. *Cyclops scutifer* and *D. longiremis* appear to be the only hypolimnetic forms in nonglacial lakes; their comparatively low frequencies in glacial lakes (Table 3) may result from interaction with hypolimnetic species such as *L. macrurus*, *S. calanoides*, *D. sicilis*, and *M. relicta*. These species show negative correlations with many predominantly epilimnetic species in glacial lakes (Table 6).

b) Species pairs which could be interacting competitively or as predator and prey are designated "b" in Table 6. Of particular interest is the possible competitive interaction between *B. longirostris* and *E. longispina* in both glacial and nonglacial lakes. *Mysis relicta* probably preys on small cladocera such as *D. longiremis* and *E. longispina* and may interact com-

TABLE 3. Fish and zooplankton frequencies, size-classes (zooplankters only), and differences in relative frequencies between glacial and nonglacial lakes.

| | Percentage of study lakes in which species occurred[a] | Size-class[b] | Frequency of occurrence | | Relative frequency[c] |
|---|---|---|---|---|---|
| | | | Glacial (total = 279 lakes) | Nonglacial (total = 249 lakes | |
| *Mysis relicta* | 37 | 6 | 193 | 0 | 1.0[d] |
| *Senecella calanoides* | 16 | 6 | 102 | 0 | 1.0 |
| *Limnocalanus macrurus* | 19 | 6 | 123 | 1 | 0.98 |
| *Diaptomus sicilis* | 15 | 4 | 94 | 3 | 0.94 |
| *Coregonus artedi* | 61 | — | 244 | 80 | 0.51 |
| *Daphnia retrocurva* | 24 | 4 | 83 | 37 | 0.38 |
| *Diaphanosoma* spp. | 50 | 2 | 169 | 86 | 0.33 |
| *Ceriodaphnia lacustris* | 12 | 2 | 37 | 19 | 0.32 |
| *Bosmina longirostris* | 53 | 1 | 176 | 93 | 0.30 |
| *Diacyclops bicuspidatus thomasi* | 52 | 3 | 202 | 130 | 0.22 |
| *Osmerus mordax* | 24 | — | 75 | 50 | 0.20 |
| *Leptodora kindtii* | 45 | 6 | 151 | 112 | 0.15 |
| *Polyphemus pediculus* | 13 | 4 | 43 | 32 | 0.15 |
| *Perca flavescens* | 88 | — | 264 | 199 | 0.14 |
| *Acanthocyclops vernalis* | 29 | 4 | 77 | 59 | 0.13 |
| *Diaptomus oregonensis* | 43 | 4 | 140 | 112 | 0.11 |
| *Epischura lacustris* | 65 | 5 | 197 | 158 | 0.11 |
| *Daphnia galeata mendotae* | 55 | 5 | 179 | 156 | 0.07 |
| *Mesocyclops edax* | 72 | 3 | 200 | 190 | 0.02 |
| *Diaptomus minutus* | 84 | 3 | 232 | 226 | 0.01 |
| *Holopedium gibberum* | 62 | 6 | 169 | 169 | 0.0 |
| *Sida crystallina* | 18 | 6 | 58 | 61 | −0.02 |
| *Chaoborus punctipennis* | 26 | 6 | 74 | 79 | −0.03 |
| *Eubosmina tubicen* | 15 | 2 | 31 | 36 | −0.07 |
| *Daphnia dubia* | 22 | 4 | 63 | 76 | −0.09 |
| *Daphnia longiremis* | 45 | 3 | 115 | 179 | −0.22 |
| *Cyclops scutifer* | 51 | 5 | 108 | 198 | −0.29 |
| *Eubosmina longispina* | 44 | 1 | 90 | 177 | −0.33 |
| *Daphnia catawba* | 19 | 3 | 33 | 68 | −0.35 |
| *Daphnia pulex* | 23 | 5 | 43 | 93 | −0.37 |
| *Alosa pseudoharengus* | 2.7 | — | 9 | 5 | — |
| *Ceriodaphnia quadrangula* | 0.7 | 2 | 2 | 1 | — |
| *Ceriodaphnia reticulata* | 1.0 | 3 | 2 | 1 | — |
| *Chaoborus flavicans* | 3.2 | 6 | 9 | 10 | — |
| *Chaoborus trivittatus* | 0.3 | 6 | 0 | 1 | — |
| *Chydorus sphaericus* | 9.8 | 1 | 25 | 22 | — |
| *Daphnia ambigua* | 4.9 | 2 | 8 | 13 | — |
| *Daphnia laevis* | 0.9 | 4 | 3 | 1 | — |
| *Daphnia parvula* | 1.9 | 2 | 3 | 2 | — |
| *Daphnia rosea* | 5.8 | 4 | 13 | 8 | — |
| *Daphnia thorata* | 0.6 | 4 | 2 | 1 | — |
| *Diaptomus ashlandi* | 2.7 | 3 | 16 | 0 | — |
| *Diaptomus birgei* | 0.4 | 4 | 0 | 2 | — |
| *Diaptomus leptopus* | 1.3 | 6 | 1 | 6 | — |
| *Diaptomus pygmaeus* | 1.4 | — | 0 | 2 | — |
| *Diaptomus sanguineus* | 6.1 | 5 | 5 | 31 | — |
| *Diaptomus spatulocrenatus* | 5.1 | 4 | 14 | 4 | — |
| *Eubosmina coregoni* | 2.2 | 2 | 4 | 2 | — |
| *Hiodon alosoides* | 5.4 | — | 26 | 3 | — |
| *Orthocyclops modestus* | 8.0 | 3 | 19 | 28 | — |

[a]For 666 lakes.
[b]Size increases from 1 to 6; see Table 4 for class boundaries.
[c]Calculated only for species occurring in more than 10% of all lakes.
[d]e.g. for *Limnocalanus*: (123−1)/(123+1) = +0.98. This index = +1 for species occurring only in glacial lakes and −1 for species occurring only in nonglacial lakes.

petitively with *C. punctipennis*. Similarly, *Leptodora kindtii* probably preys on *B. longirostris* in nonglacial lakes. Cladoceran pairs such as *Daphnia pulex*−*Diaphanosoma* spp. and *Daphnia dubia*−*D. retrocurva* may be interacting

TABLE 4. Correlations of zooplankton size categories with components 1 through 3 from ecological PCA. (Correlations greater than 0.08 are significant at $P \leq 0.05$.)

| | Principal component | | |
|---|---|---|---|
| Size-class (mm) | 1 | 2 | 3 |
| 1 (<0.7) | −0.02 | −0.35 | 0.73 |
| 2 (0.7−0.99) | 0.79 | 0.09 | −0.38 |
| 3 (1.0−1.29) | 0.24 | 0.17 | 0.15 |
| 4 (1.3−1.59) | 0.80 | 0.31 | 0.40 |
| 5 (1.6−2.0) | −0.15 | 0.05 | 0.18 |
| 6 (>2.0) | 0.56 | −0.82 | −0.07 |

competitively. Alternatively, some of these negative correlations may simply reflect the different times of maximum seasonal abundance of the various species.

c) Possible instances of planktivorous fish preying on plankters are designated "c" in Table 6. Thus, in nonglacial lakes smelt may be preying on the comparatively large species *Daphnia galeata mendotae*, *Diaphanosoma* spp., and *H. gibberum* while cisco may be preying on the large *D. pulex*. This is consistent with observations made by Dadswell who, while sampling lakes barren of smelt, cisco, and *M. relicta* outside former glacial lake boundaries (Dadswell 1972, 1974, 1975), repeatedly observed abundant large cladocerans whereas inside the boundary they were not observed. In glacial lakes positive correlations with planktivorous fish such as those shown by the small *D. longiremis* and *Ceriodaphnia lacustris* could reflect competitive release resulting from depression of larger cladocerans (e.g. Lynch 1979). The positive correlation between the predatory *M. relicta* and cisco could be an example of predatory enhancement as suggested by Dodson (1970).

TABLE 5. Spearman rank correlations of ecological components with limnological variables (footnotes as in Table 2).

| | Principal component | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Influence | 0.30 | 0.02 | 0.14 |
| Basin area ($km^2$) | 0.19 | −0.11 | 0.00 |
| Color (mg/L) | 0.17 | 0.00 | −0.19 |
| Max. hypolimnetic temp. (°C) | 0.16 | −0.07 | −0.06 |
| Modified Ryder's index | 0.15 | 0.31 | 0.03 |
| Surface area (ha) | 0.10 | −0.19 | 0.05 |
| Epilimnion pH | 0.08 | 0.26 | 0.06 |
| Depth (m) below which $[O_2] < 2$ mg/L | 0.08 | 0.23 | 0.01 |
| Total hardness (mg/L) | 0.04 | 0.32 | 0.10 |
| Lake volume ($m^3$) | 0.02 | −0.19 | 0.08 |
| Elevation (m) | −0.06 | −0.29 | −0.02 |
| Maximum depth (m) | −0.06 | −0.07 | 0.10 |
| Hypolimnion pH | −0.09 | 0.23 | 0.09 |
| Secchi depth (m) | −0.17 | −0.13 | 0.22 |
| Mean depth (m) | −0.19 | −0.05 | 0.09 |

TABLE 6. Interspecies correlations (Spearman's rank) among zooplankters and fish for glacial and nonglacial lakes.

| Nonglacial lakes \ Glacial | Lm | Sca | El | Dm | Do | Ds | Me | Cs | Av | Dbt | Dr | Dl | Dgm | Dp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *L. macrurus* | | 0.00 | −0.13[a] | −0.13[a] | −0.03 | 0.12 | −0.07 | −0.22[b] | 0.10 | −0.05 | −0.03 | −0.02 | −0.02 | 0.06 |
| *S. calanoides* | —[e] | | 0.02 | −0.01 | −0.06 | 0.10 | 0.09 | 0.02 | −0.10 | −0.07 | 0.12 | −0.05 | 0.06 | −0.18[a] |
| *E. lacustris* | — | — | | 0.25 | 0.06 | −0.03 | 0.05 | −0.02 | −0.08 | −0.00 | 0.10 | 0.02 | 0.21 | −0.03 |
| *D. minutus* | — | — | −0.16[b] | | 0.04 | 0.01 | 0.08 | −0.02 | 0.01 | 0.12 | 0.04 | 0.02 | 0.14 | −0.05 |
| *D. oregonensis* | — | — | 0.17 | 0.02 | | −0.15[a] | 0.34 | −0.03 | 0.20 | 0.14 | 0.26 | 0.05 | 0.18 | −0.08 |
| *D. sicilis* | — | — | — | — | — | | −0.14[a] | −0.10 | −0.07 | 0.03 | −0.04 | −0.13[b] | 0.01 | −0.05 |
| *M. edax* | — | — | 0.20 | −0.01 | 0.12 | — | | −0.10 | 0.10 | 0.15 | 0.28 | −0.07 | 0.19 | 0.03 |
| *C. scutifer* | — | — | −0.22[a] | 0.07 | 0.00 | — | −0.10 | | −0.23[a] | −0.22[a] | −0.12[a] | 0.05 | −0.17[a] | 0.07 |
| *A. vernalis* | — | — | 0.08 | 0.10 | 0.07 | — | 0.10 | −0.13[d] | | 0.08 | 0.16 | 0.01 | 0.16 | 0.01 |
| *D.b. thomasi* | — | — | 0.25 | −0.10 | 0.41 | — | 0.13 | −0.23[a] | 0.10 | | 0.15 | 0.18 | 0.14 | 0.03 |
| *D. retrocurva* | — | — | 0.17 | −0.04 | 0.17 | — | 0.14 | −0.19[d] | 0.05 | 0.17 | | 0.11 | 0.01 | −0.22[b] |
| *D. longiremis* | — | — | 0.11 | 0.02 | 0.23 | — | 0.12 | 0.02 | 0.12 | 0.08 | 0.17 | | −0.08 | 0.07 |
| *D.g. mendotae* | — | — | 0.32 | −0.10 | 0.30 | — | 0.27 | −0.19[a] | 0.01 | 0.35 | 0.09 | 0.10 | | −0.01 |
| *D. pulex* | — | — | 0.01 | −0.12[b] | 0.03 | — | 0.07 | 0.05 | −0.02 | 0.11 | −0.10 | −0.03 | 0.17 | |
| *D. dubia* | — | — | 0.28 | −0.07 | 0.23 | — | 0.17 | −0.06 | 0.10 | 0.25 | −0.10 | 0.15 | 0.18 | 0.06 |
| *D. catawba* | — | — | 0.02 | −0.02 | −0.03 | — | 0.07 | −0.09 | −0.02 | −0.02 | −0.04 | −0.08 | 0.01 | −0.06 |
| *L. kindtii* | — | — | 0.43 | −0.17[b] | 0.26 | — | 0.17 | −0.14[a] | 0.04 | 0.17 | 0.17 | 0.20 | 0.23 | 0.03 |
| *P. pediculus* | — | — | 0.06 | 0.02 | −0.03 | — | 0.09 | −0.15[a] | 0.06 | 0.07 | 0.23 | 0.07 | −0.02 | −0.07 |
| *S. crystallina* | — | — | 0.19 | −0.08 | 0.06 | — | 0.07 | −0.15[a] | 0.04 | 0.11 | 0.05 | 0.05 | 0.15 | −0.06 |
| *Diaphanosoma* spp. | — | — | 0.31 | −0.04 | 0.36 | — | 0.25 | −0.09 | 0.16 | 0.33 | 0.31 | 0.31 | 0.24 | −0.11[b] |
| *C. lacustris* | — | — | −0.01 | 0.03 | 0.13 | — | 0.03 | −0.09 | 0.16 | 0.17 | 0.25 | 0.08 | 0.01 | −0.13[d] |
| *H. gibberum* | — | — | 0.28 | 0.02 | −0.00 | — | 0.09 | −0.12[a] | 0.00 | −0.05 | 0.11 | 0.18 | 0.12 | −0.09 |
| *B. longirostris* | — | — | 0.02 | 0.01 | −0.06 | — | 0.06 | −0.11[a] | 0.11 | 0.04 | 0.02 | −0.10 | −0.09 | −0.02 |
| *E. longispina* | — | — | 0.18 | 0.03 | 0.06 | — | 0.01 | 0.03 | −0.09 | −0.01 | −0.00 | 0.09 | 0.03 | −0.07 |
| *E. tubicen* | — | — | −0.02 | 0.02 | 0.11 | — | 0.06 | −0.05 | 0.09 | 0.04 | 0.11 | −0.04 | −0.08 | −0.19[d] |
| *C. punctipennis* | — | — | 0.23 | −0.07 | 0.35 | — | 0.25 | −0.15[b] | 0.17 | 0.27 | 0.17 | 0.22 | 0.22 | 0.02 |
| *M. relicta* | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| *O. mordax* | — | — | −0.11[c] | −0.03 | −0.13[c] | — | −0.02 | −0.01 | 0.10 | 0.04 | 0.02 | −0.09 | −0.13[c] | −0.08 |
| *C. artedi* | — | — | 0.09 | 0.03 | 0.14 | — | 0.08 | −0.10 | 0.04 | 0.10 | 0.04 | 0.04 | 0.01 | −0.13[c] |
| *P. flavescens* | — | — | −0.12[c] | −0.07 | 0.10 | — | 0.09 | −0.10 | −0.03 | 0.03 | 0.04 | 0.03 | −0.03 | −0.05 |

[a]Probable epilimnetic vs. hypolimnetic pairs.
[b]Probable predator−prey or competitor pairs.
[c]Probably fish−zooplankter predator pairs.
[d]Correlations possibly spurious because of discrepancies in frequencies of occurrence of the two species.
[e]— indicates a correlation coefficient cannot be determined.

### Cogeneric and Cofamilial Occurrences

On the basis of a null hypothesis (Connor and Simberloff 1979) of random assortment, the number of lakes expected to contain a particular association of related species will be given by the product of the frequencies of the component species (Table 3) divided by the total number of lakes. Departure from expectation provides a basis for further investigation into the exact geographical distribution of the component species and/or physicochemical and biological factors affecting the species. Only a small subset of more interesting associations is considered here.

Within the genus *Daphnia* a maximum of six species (all obtained in a single net haul) inhabit a lake, with six such combinations occurring (Table 7), all much more frequent than expected by chance. Apparently the only other such association reported in North America is for Aziscoos Lake, Maine (Tappa 1965) (identical with the second *Daphnia* association in Table 7). Interestingly the six lakes involved are located in the Gatineau area of western Quebec, a region of confluence of major geological influences (Dadswell 1974) (possibly unstable) and two of the six lakes are reservoirs (possibly young ecosystems). Combinations of five and fewer *Daphnia* species occurred generally as expected on the basis of random assortment.

Within the Bosminidae combinations of two and three species occurred as expected by chance except for the pair *Eubosmina coregoni* and *Eubosmina tubicen*, which was expected but not observed. There is evidence, however, that these species are restricted to hard or moderately hard and soft waters, respectively (Carter 1971; Carter et al. 1980).

The only pair of *Diaptomus* species expected but observed neither by us nor others (Rigler and Langford 1967; Turvey 1967) was *D. sicilis* and *Diaptomus sanguineus*. Given the clear geographic and ecological overlap of these species (Carter et al. 1980), it seems possible they may interact in some manner. Rigler and Langford (1967) and Turvey (1967) only found our first three associations of *Diaptomus* species but neither of the four species associations (the largest we found), probably because of their less extensive surveys. No unexpected combinations of cyclopoid copepod species were found.

## General Discussion and Conclusions

The dominant patterns of community structure in this study are related to the glacial history of the region. A number of species, particularly the large facultative predators *M. relicta*, *S. calanoides*, and *L. macrurus* (Bowers and Warren 1977; Lasenby and Langford 1973; Powell personal communication), have quite disjunct distributions between glacial and nonglacial lakes. Some of the community patterns observed are consistent with the predatory and competitive interactions among zooplankters reported by others in small lakes and ponds (e.g. Hall et al. 1976) but generally the expected high correlations among species are lacking. Although this may result partly from the heterogeneous nature of our study area, it is also possible that the biological inter-

Only species occurring in more than 10% of lakes are included. (All coefficients >0.11 are significant at $P \leq 0.05$.)

| Lakes | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dd | Dc | Lk | Pp | Scr | Dsp | Cl | Hg | Bl | El | Et | Cp | Mr | Om | Ca | Pf |
| 0.13 | −0.08 | −0.06 | 0.02 | 0.05 | −0.09 | 0.05 | 0.02 | −0.08 | 0.18 | −0.11[a] | 0.00 | 0.06 | 0.16 | 0.03 | 0.01 |
| 0.09 | −0.07 | 0.18 | 0.12 | 0.14 | 0.20 | −0.10 | 0.19 | 0.16 | −0.03 | 0.17 | 0.01 | 0.29 | 0.17 | 0.21 | 0.07 |
| 0.08 | 0.04 | 0.31 | 0.05 | 0.14 | 0.19 | −0.20[d] | 0.25 | 0.09 | −0.01 | 0.03 | −0.00 | −0.04 | −0.21[c] | 0.07 | 0.08 |
| 0.06 | −0.07 | 0.04 | −0.03 | 0.00 | 0.22 | 0.01 | 0.11 | 0.11 | −0.05 | 0.03 | −0.08 | 0.10 | −0.07 | 0.18 | 0.12 |
| 0.06 | −0.01 | 0.17 | 0.02 | 0.11 | 0.34 | 0.18 | −0.08 | −0.02 | −0.06 | 0.06 | 0.22 | −0.02 | 0.04 | 0.06 | 0.05 |
| −0.01 | −0.05 | −0.11[a] | −0.03 | −0.11[a] | −0.06 | −0.05 | −0.16[a] | 0.02 | −0.08 | −0.02 | −0.14[a] | 0.42 | 0.08 | 0.15 | 0.07 |
| 0.01 | 0.04 | 0.23 | 0.16 | 0.12 | 0.30 | 0.16 | 0.13 | −0.04 | −0.08 | 0.07 | 0.20 | 0.02 | −0.01 | 0.01 | −0.01 |
| −0.09 | −0.04 | −0.03 | −0.09 | −0.01 | −0.16[a] | −0.04 | −0.02 | −0.04 | 0.15 | −0.01 | −0.01 | −0.11[b] | −0.06 | −0.02 | −0.00 |
| 0.18 | 0.06 | 0.02 | −0.00 | 0.03 | 0.13 | 0.11 | −0.17[b] | 0.05 | −0.01 | −0.11[b] | −0.01 | 0.03 | 0.07 | 0.07 | 0.01 |
| 0.07 | −0.05 | 0.04 | 0.05 | 0.02 | 0.08 | 0.11 | −0.07 | 0.15 | −0.04 | −0.04 | 0.01 | −0.01 | 0.03 | −0.00 | 0.02 |
| −0.19[b] | −0.08 | 0.24 | 0.06 | 0.05 | 0.38 | 0.26 | 0.13 | 0.11 | −0.06 | 0.07 | 0.07 | −0.03 | 0.13 | 0.11 | −0.02 |
| −0.02 | 0.00 | 0.11 | 0.10 | 0.09 | −0.04 | 0.16 | 0.06 | 0.07 | 0.20 | 0.19 | −0.06 | −0.39[b] | 0.27 | −0.02 | −0.03 |
| 0.20 | 0.06 | 0.22 | 0.09 | 0.17 | 0.29 | −0.05 | −0.05 | 0.03 | −0.10 | −0.03 | 0.10 | 0.08 | −0.09 | 0.13 | 0.12 |
| −0.02 | 0.20 | −0.13[d] | −0.04 | −0.07 | −0.20[d] | 0.05 | −0.12[d] | −0.18[d] | 0.08 | −0.12[b] | 0.07 | −0.28[d] | −0.03 | −0.15[d] | 0.01 |
| | 0.10 | 0.20 | 0.03 | 0.37 | 0.17 | −0.07 | −0.01 | −0.00 | 0.16 | 0.01 | 0.02 | 0.00 | −0.06 | 0.00 | 0.03 |
| 0.08 | | 0.11 | 0.08 | 0.07 | 0.01 | 0.01 | 0.05 | −0.06 | 0.05 | 0.01 | 0.18 | −0.12[d] | −0.04 | −0.13[d] | −0.00 |
| 0.23 | 0.08 | | 0.19 | 0.32 | 0.33 | −0.02 | 0.22 | −0.01 | 0.18 | 0.15 | 0.09 | −0.02 | 0.09 | 0.09 | 0.09 |
| 0.14 | 0.08 | 0.09 | | 0.21 | 0.16 | 0.06 | 0.27 | 0.01 | 0.06 | 0.20 | 0.15 | −0.08 | 0.18 | 0.02 | 0.07 |
| 0.33 | 0.26 | 0.20 | 0.17 | | 0.24 | −0.13[b] | 0.19 | 0.05 | 0.19 | 0.10 | 0.05 | −0.05 | −0.08 | 0.06 | 0.01 |
| 0.31 | 0.01 | 0.37 | 0.33 | 0.23 | | 0.13 | 0.15 | −0.01 | −0.12[b] | 0.11 | 0.21 | 0.09 | 0.08 | 0.15 | 0.12 |
| −0.02 | −0.07 | 0.00 | 0.22 | −0.01 | 0.20 | | −0.04 | −0.10 | −0.13[b] | 0.02 | 0.18 | −0.02 | 0.29 | −0.07 | −0.07 |
| 0.13 | 0.07 | 0.26 | 0.17 | 0.15 | 0.21 | 0.00 | | 0.11 | 0.19 | 0.22 | 0.09 | −0.10 | 0.05 | 0.02 | −0.01 |
| −0.08 | −0.16[b] | −0.14[b] | 0.05 | −0.07 | −0.05 | 0.05 | 0.01 | | −0.25[b] | −0.04 | −0.18[b] | 0.09 | 0.02 | 0.09 | 0.18 |
| 0.14 | 0.04 | 0.11 | −0.06 | 0.12 | 0.05 | −0.14[b] | 0.13 | −0.37[b] | | 0.09 | −0.09 | −0.28[ab] | −0.01 | −0.10 | −0.05 |
| 0.04 | 0.07 | 0.01 | 0.18 | 0.04 | 0.19 | 0.17 | 0.15 | 0.08 | −0.06 | | 0.16 | −0.03 | 0.09 | 0.07 | 0.06 |
| 0.19 | 0.12 | 0.24 | 0.05 | 0.16 | 0.40 | 0.19 | 0.09 | −0.03 | −0.00 | 0.14 | | −0.11[ab] | 0.05 | 0.01 | 0.02 |
| — | — | — | — | — | — | — | — | — | — | — | — | | −0.06 | 0.29 | 0.09 |
| −0.03 | −0.08 | −0.07 | −0.04 | −0.13[c] | −0.14[c] | 0.05 | −0.25[c] | −0.02 | −0.05 | −0.07 | −0.04 | — | | 0.07 | 0.01 |
| 0.08 | 0.07 | 0.16 | 0.10 | 0.09 | 0.19 | 0.10 | −0.01 | 0.08 | −0.02 | 0.14 | 0.16 | — | −0.09 | | 0.30 |
| 0.11 | 0.02 | 0.09 | 0.02 | −0.09 | 0.08 | 0.11 | −0.09 | 0.09 | −0.13[c] | 0.09 | 0.06 | — | 0.05 | 0.28 | |

TABLE 7. Associations of six *Daphnia* species, two and three bosminid species, and three and four *Diaptomus* species and their frequency of occurrence.

| | No. of lakes | | | | | |
|---|---|---|---|---|---|---|
| *Daphnia* spp. | 1 | 1 | 1 | 1 | 1 | 1 |
| *D. retrocurva* | × | × | × | × | × | |
| *D. longiremis* | × | × | × | × | | × |
| *D. galeata mendotae* | × | × | × | × | × | × |
| *D. ambigua* | × | × | × | | × | × |
| *D. pulex* | × | | | × | | × |
| *D. parvula* | | | | | × | |
| *D. dubia* | × | × | | × | | × |
| *D. catawba* | | × | × | × | × | × |
| *D. laevis* | | | | | × | |
| *D. rosea* | | | × | | | |
| *D. thorata* | | | | | | |

| | No. of lakes | | | | | |
|---|---|---|---|---|---|---|
| Bosminidae | 95 | 62 | 8 | 51 | 2 | 25 | 2 |
| *Bosmina longirostris* | × | × | × | | | × | × |
| *Eubosmina longispina* | × | | | × | × | × | × |
| *E. tubicen* | | × | | × | | × | |
| *E. coregoni* | | | × | | × | | × |

| | No. of lakes | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Diaptomus* spp. | 15 | 3 | 30 | 6 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 3 | 1 | 1 |
| *Diaptomus minutus* | × | × | × | × | × | × | × | × | × | × | × | × | | | × | × |
| *D. oregonensis* | × | × | × | × | × | × | × | | | | | | × | × | × | × |
| *D. sanguineus* | × | | | | | | | × | × | | | | × | | × | |
| *D. ashlandi* | | × | | | | | | | | × | | | | × | | × |
| *D. sicilis* | | | × | | | | | | | × | × | | | × | | × |
| *D. spatulocrenatus* | | | | × | | | | × | | | × | × | | | | |
| *D. leptopus* | | | | | × | | | | | | | | | | | |
| *D. birgei* | | | | | | × | | | × | | | | × | | × | |
| *D. reighardi* | | | | | | | × | | | | | | | | | |
| *D. pygmaeus* | | | | | | | | | | | | × | | | | |

actions observed in small lakes and ponds may be less intense in the larger, more physically complex aquatic ecosystems examined here, where separation in time and space is more probable. This cautions against the uncritical application of zooplankton community theory developed from simple ecosystems to the larger lakes characteristic of the vast glaciated regions of North America. Species interactions undoubtedly exist in larger lakes but they may not result in exclusion and are thus difficult to defend solely on the basis of sampling. Only strong interactions, such as the combined impact of the large glacial opportunists, are likely to have substantial effects on community structure. There appear to be more "subtle mechanisms operating to structure zooplankton communities" than have generally been recognized (Dodson 1979; O'Brien et al. 1979).

More data are required to evaluate the contention that clear patterns in zooplankton species associations exist only within geographically homogeneous regions. In particular, experimental evidence is necessary to evaluate the following: our suggestion about the "controlling" influence of the hypolimnetic predators; some of the stronger negative correlations between species; possible competitive interaction between *D. sicilis* and *D. sanguineus*; and the significance of co-occurrences of six *Daphnia* species.

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# Larval and Juvenile Stages of the Isopod *Holophryxus alaskensis* (Epicarida, Dajidae) Parasitic on Decapods[1]

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COYLE, K. O., AND G. J. MUELLER. 1981. Larval and juvenile stages of the isopod *Holophryxus alaskensis* (Epicarida, Dajidae), parasitic on decapods. Can. J. Fish. Aquat. Sci. 38: 1438 – 1443.

Complete descriptions of the epicarid, microniscid, cryptoniscid, and juvenile stages of *Holophryxus alaskensis* are given. The epicarid differs from other epicarids by its chelate pereopods. The intermediate host is *Euchaeta elongata* (Copepoda). The cryptoniscid stage differs from others of the Dajidae in lacking a buccal sucker and posterior projection on article 1 of antenna 1. The cryptoniscid attaches to the body wall beneath the carapace of *Pasiphaea pacifica* (Decapoda), where it metamorphoses to the juvenile stage before migrating to the dorsal side of the carapace.

*Key words*: Isopoda, Epicarida, Dajidae, cryptoniscid, epicarid, microniscid, parasite, Decapoda

COYLE, K. O., AND G. J. MUELLER. 1981. Larval and juvenile stages of the isopod *Holophryxus alaskensis* (Epicarida, Dajidae), parasitic on decapods. Can. J. Fish. Aquat. Sci. 38: 1438 – 1443.

L'article qui suit contient une description complète des stades épicaridien, microniscus, cryptoniscus et juvénile de *Holophryxus alaskensis*. Le stade épicaridien diffère de celui des autres épicarides par ses péréiopodes chélatés. L'hôte intermédiaire est *Euchaeta elongata* (Copepoda). Le stade cryptoniscus diffère de celui des autres Dajidae par l'absence d'une ventouse buccale et d'une projection postérieure sur l'article 1 de l'antenne 1. Le stade cryptoniscus se fixe à la paroi du corps sous la carapace de *Pasiphaea pacifica* (Decapoda), où il se transforme en juvénile avant d'émigrer à la partie dorsale de la carapace.

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WHILE examining the shrimp in the aquatic collections of the University of Alaska Museum, we noticed an unusually large number of *Pasiphaea pacifica* Rathbun (1902) that were parasitized by the epicarid parasite *Holophryxus alaskensis* Richardson (1905), with several of the adult specimens having unmetamorphosed cryptoniscid stage males in the brood pouch. This allowed us to identify the cryptoniscid larvae from plankton samples taken in the same area, and trace the development back through the epicarid stage. The following paper presents descriptions and diagrams of the larval and juvenile stages of *Holophryxus alaskensis*, and a short discussion of their relationship to other members of the family Dajidae.

When *H. alaskensis* was first described by Richardson (1905), the host and male were not known. Later, Richardson (1908) described another species, *Holophryxus californiensis*, a parasite of *P. pacifica*. Nierstrasz and Brender à Brandis (1931) described the species *Hypodajus georgiensis*, also a parasite of *P. pacifica* from the Straits of Georgia, British Columbia. Butler (1964) synonymized the two under *H. alaskensis* and redescribed the adults and late juvenile stage. We follow Butler (1964) in considering *H. georgiensis* and *H. californiensis* to be synonymous with *H. alaskensis*, which has previously been reported from Santa Barbara Channel, California, to Behm Canal, Alaska (Butler 1964). This paper extends its range to Prince William Sound, Alaska.

## Methods

All specimens for this study were collected from Knight Island Passage, Prince William Sound, Alaska (60°15′N, 148°00′W). The collections were made in March 1979, July 1979, and December 1980, and larval stages were found in the zooplankton on all three occasions. The zooplankton was collected with a 1-m ring net (202-μm mesh size) towed vertically from about 500 m in depth to the surface. The juvenile and adult shrimp were collected with a 2-m Tucker trawl (2-mm mesh size), an Isaacs Kidd trawl, and a bongo net (505-μm mesh size) towed at night in the upper 100 m. All specimens were fixed in 10% Formalin and museum specimens were transferred to 40% isopropynol. To determine the

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intermediate host, it was necessary to examine live material, as the microniscid stage falls off the host after preservation.

## Results

The following is a description of the dajid epicarid stage larvae from our plankton samples. Because *H. alaskensis* was the only dajid parasite in our samples, because this species was the overwhelmingly dominant isopod of the suborder Epicarida (there were no other epicarid stage dajids found in the plankton) and because this occurred consistently where *H. alaskensis* was found, we are confident in assigning these epicarid stage isopods to *H. alaskensis*.

### The Epicarid Stage (Fig. 1, A–G)

Length 0.2–0.3 mm. Head about 25% of total body length, anterior edge rounded; coxae pointed, anterior edges with two small denticles (Fig. 1A, F). Buccal cone directed anteriorly, mandibles with jagged tips; antenna 1 of two articles, basal article somewhat broad with three bifurcate setae inward from flagellum and one bifurcate seta lateral to flagellum, which is

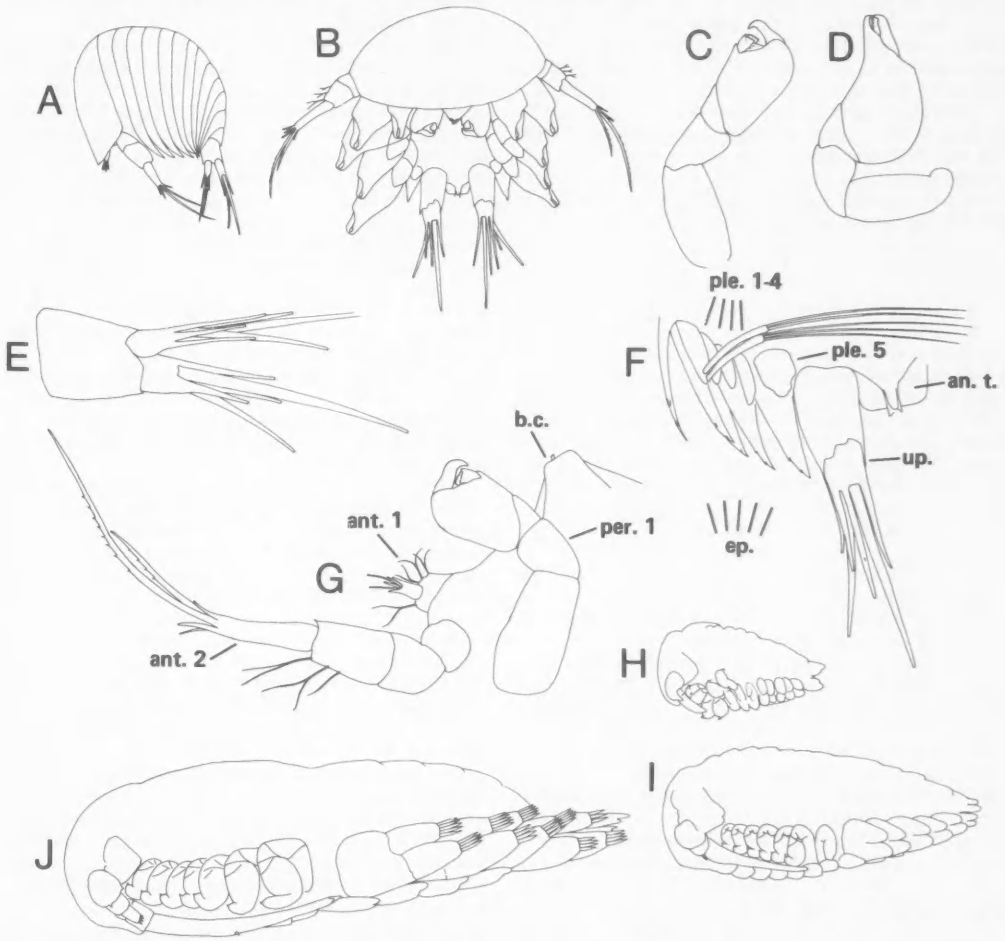


Fig. 1. *Holophryxus alaskensis*, (A–G) epicarid stage, length 0.23 mm; (H–J) microniscid. (A) lateral view; (B) anterior view; (C) pereopod 1; (D) pereopod 2, (E) uropod, lateral view; (F) right posterior quarter, showing epimeral plates (ep.), pleopods 1–4 (ple. 1–4), rami on pleopods 2–4 omitted, pleopod 5 (ple. 5), uropod (up.) and anal tube (an. t.); (G) right ventral side of head showing buccal cone (b.c.), antenna 1 (ant. 1), antenna 2 (ant. 2), pereopod 1 (per. 1); (H) microniscid stage 1 length 0.4 mm; (I) stage 2, 0.8 mm; (J) stage 3, 1.2 mm.

of one article crowned with about seven spines of unequal length; antenna 2 of four articles, third article with three lateral setae, the distal two are bifurcate, fourth article terminating in five spines, two being much longer than the others and having serrate lateral edges (Fig. 1B, G). Pereopod 1 (Fig. 1B, C, G) subchelate, articles 3 and 4 apparently coalesced, palm of article 6 oblique, palmar edge with curved serrate plate; dactyl unguiform, reaching end of palm. Pereopods 2–5 (Fig. 1B, D) similar to each other, chelate, articles 3 and 4 apparently coalesced, article 6 oval, thumb with serrate inner edge; dactyl unguiform, reaching end of thumb. Four pairs of pleopods present, all biramus, rami long and narrow, bandlike, inner ramus with two, outer ramus with three long plumose setae; fifth pair of pleopods reduced to short broad bump (Fig. 1F). Uropods biramous, rami situated dorsoventrally to one another (Fig. 1A, E), rami ending in five or six long stout spines. Anal tube short (Fig. 1B, F).

The epicarid lacks eyes and distinct coloration. It attaches to stage V of the copepod *Euchaeta elongata* Easterly (1913), on the side of the cephalathorax, with its head directed anteriorly as illustrated in Sars (1899, Plate 92, Microniscus, transitory stage of Epicarida). It appears to hold on mainly by the first pair of pereopods.

### The Microniscid Stage (Fig. 1, H–J)

The microniscid stages (Fig. 1, H–J) lack eyes, the cuticle is thin and flacid, articulation and segmentation are reduced or absent. The development of pereopod 7, the pleopods, uropods, and antenna 2 can be followed with each stage. The animals are weakly attached to the host and are easily knocked off. They are immotile and presumably die if knocked off before completion of metamorphosis to the cryptoniscid stage. The fully developed cryptoniscid stage can be seen beneath the cuticle of the last microniscid stage.

### The Cryptoniscid Stage (Fig. 2, A–N)

Length 1.2 mm. Head with rounded anterior edge, eyes absent, buccal cone directed anteriorly, coxa 1–7 lacking teeth (Fig. 2A). Mandibles long, ends sagitate (Fig. 2D). Antenna 1 (Fig. 2B) article 1 lacking spines or teeth, edges evenly rounded, two small setae present near anterior edge, one in center, two near posterior edge; article 2 lacking spines or teeth, two setae near anterior edge, 1 seta near posterior edge; article 3 with numerous esthetascs, two flagella present, one with two terminal setae, one with numerous terminal and subterminal setae. Antenna 2 of nine articles (Fig. 2C), peduncle of four articles, peduncle articles 2-4 with one terminal seta; flagellar articles with two distal setae, last flagellar article crowned by three long setae. Pereopod 1 (Fig. 2E) short; articles 2, 3, and 6 of about equal length; article 4 with short anterior lobe terminating in small seta; article 5 terminating in one to several sharp teeth, article 6 with oblique palmar edge; dactyl short and thick, reaching to end of palm. Pereopods 2–6 (Fig. 2, F–J) similar to pereopod 1, but gradually increase in size, palms with two transverse rows of one to several spines. Pereopod 7 (Fig. 2K) with articles 2 and 3 of equal length; article 4 about two-thirds the length of article 3, posterior lobe ends in one to two short spines; article 5 about half the length of article 3, distal end with several stout spines; article 6 slightly shorter than 3, palmar edge with two transverse rows of spines; article 7 exceeds palm by one-third its length and tapers evenly to a point. Pleopods 1–4 (Fig. 2M) similar in shape, inner edge of sympod with two long setae having trifid tips; inner ramus with five, outer ramus with four, long plumose setae. Pleopod 5 (Fig. 2N) somewhat narrower than pleopods 1–4; inner ramus with three, outer ramus with four long plumose setae. Uropods (Fig. 2L) with short peduncle, ventral distal edge and lateral distal edge with stout spine; outer ramus slightly shorter than inner ramus, distal end with three stout spines of uneven length; inner ramus with two stout distal spines of uneven length and two minute setae near the dorsal proximal end. Pleotelson with truncate distal end.

After finding a host, the cryptoniscid crawls beneath the carapace and attaches with the head oriented against the body wall just dorsal of the gills, and although they can probably attach to fully grown shrimp, we have found them only on juveniles of about 20 mm total length. The cryptoniscids found beneath the carapace are morphologically identical with those found in the brood pouch of the adult female isopods.

After attaching to the host, the cryptoniscid undergoes metamorphosis to the juvenile stage (Fig. 2O). The pleopods, uropods, and abdominal segmentation are lost. Pereopod 7 is lost, with only the coxa remaining, the first body segment fuses to the head, antenna 1 is reduced to an asetose bumplike structure, and articulation of the flagellum and peduncle of antenna 2 is lost, with only the articulation line between the flagellum and peduncle remaining. Pereopods 1–6 take the shape of the adult pereopods, with articulation lines weakened or absent (Fig. 2T). The juveniles beneath the carapace varied in length from 1.7 to 4.0 mm. Up to 30% of the shrimp in our catches were infected with the juvenile parasite, with up to three parasites per shrimp.

After reaching the juvenile stage, the parasite moves from beneath the carapace, up the side of the first abdominal segment, and onto the dorsal side of the carapace where it attaches to the area above the pericardium. Pereopods 1–5 form a row of five hooks on each side of the body, which, when contracted inward, dig into the side of the carapace, securely fastening the parasite to its host (Fig. 2Q, T).

Final metamorphosis to the adult involves loss of coxa 7, formation of brood plates on pereopods 1–5, and lengthening of the area between pereopods 5 and 6, with loss of pereopod 6 and reduction of coxa 6 to a small bump (Fig. 2P, Q). The mandible changes from the sagitate form in the cryptoniscid stage (Fig. 2D) to a rasplike organ (Fig. 2R), which drills a hole in the host's carapace. Breeding does not occur until the brood pouch is formed. The morphology of the adult females and males has been fully described elsewhere (Nierstrasz and Brender à Brandis 1931; Butler 1964) and will not be repeated here, except to mention that what has been called a maxilliped is the peduncle of antenna 2 (Fig. 2S), which expands to form a lobe around the buccal cone.

## Discussion

Although earlier authors (Bonnier 1900; Tattersall 1911; Nierstrasz and Brender à Brandis 1926) considered the dajid epicarids to have uniramous uropods and pleopods; our material and that of Taberly (1954a) contradict this. Part of the

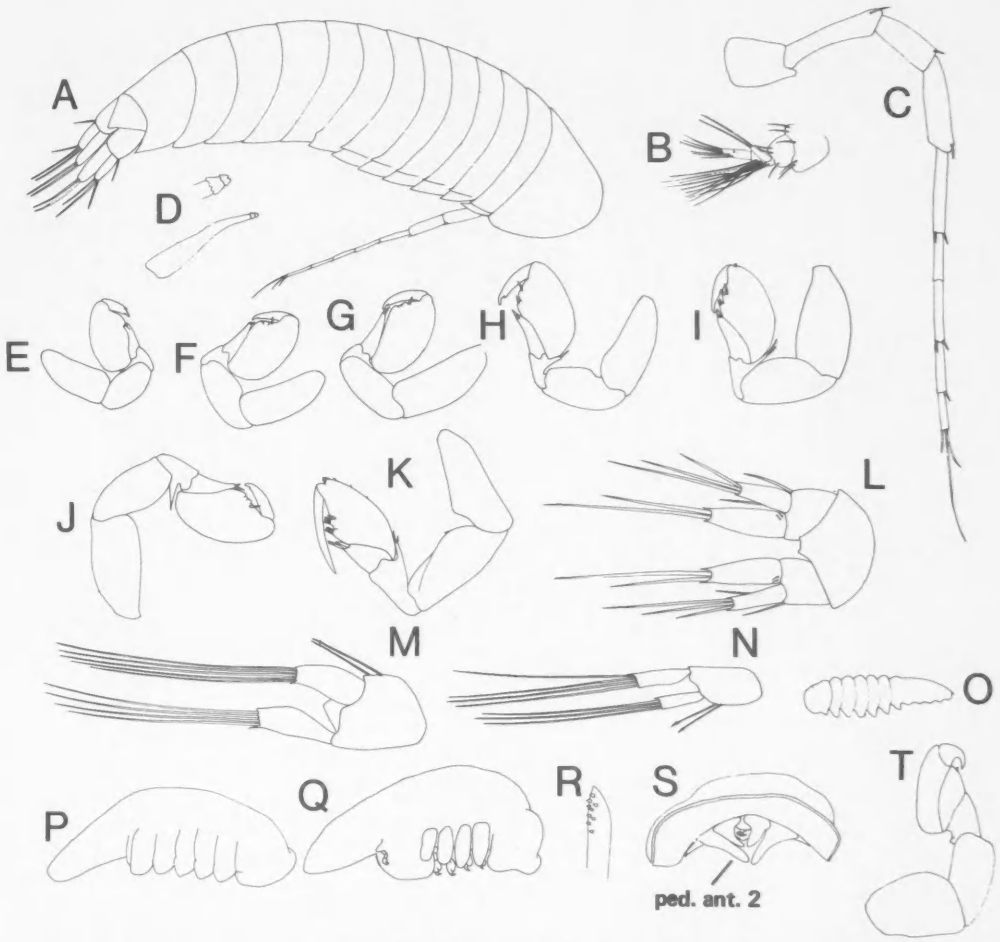


FIG. 2. *Holophryxus alaskensis*, (A–N) cryptoniscid stage, length 1.2 mm; (O–T) juvenile. (A) dorsal lateral view; (B) antenna 1; (C) antenna 2; (D) mandible; (E–K) pereopods 1–7; (L) uropods and pleotelson; (M) pleopod 1; (N) pleopod 5; (O) juvenile, length 1.8 mm; (P) juvenile, length 3.0 mm; (Q–T) juvenile, 5.0 mm; (Q) lateral view; (R) tip of mandible; (S) head, ventral view, showing peduncle of antenna 2 (ped. ant. 2) forming lobe around buccal cone; (T) pereopod 1.

confusion may arise from the small size of epicarid stage larvae (0.2–0.3 mm for *H. alaskensis*), which makes manipulation and observation difficult, and from the unusual dorsoventral orientation of the uropod rami, which obscures the biramous condition from dorsal and ventral views. We suspect that a re-examination of the dajid epicarids thus far described might show them to have biramous uropods and pleopods. The thick spines on the rami of the uropods have thus far been found only on dajids.

The epicarid stage of *H. alaskensis* is most easily distinguished from those of other dajids by the chelate condition of pereopods 2–5, the other epicarids having subchelate or simple pereopods. We don't know whether this is a specific, generic, or subfamily character. Although Stephensen (1912) described an epicarid stage larvae from the brood pouch of *Holophryxus acanthephyrae* Stephensen, it was only partially developed, and the condition of the fully developed pereopods could not be determined. Stephensen's epicarid probably represents what Gilson (1909) referred to as the "epicaridium achéte." All other epicarid stage dajids so far described are from those genera which parasitize mysids.

The microniscid stage of *H. alaskensis* is not very different from those described and diagramed by earlier authors (Sars 1899; Bonnier 1900; Tattersall 1911). Microniscids tend to be nondescript, lacking the spines and setation commonly used to distinguish closely related peracarids. Since the mi-

croniscid is not motile, metamorphosis from epicarid to cryptoniscid must be completed before the host molts. A host specimen, which was parasitized by a final stage microniscid, was kept in the laboratory for several days and swam vigorously showing no obvious behavioral abnormalities. The intermediate host, *E. elongata*, has been reported from the western Pacific, Bering Sea, and eastern Pacific south to California (Brodskii 1950, as *Pareuchaeta japonica*). Its range, therefore, overlaps that of the final host.

Previous authors (Sars 1899, Bonnier 1900; Tattersall 1911; Nierstrasz and Brender à Brandis 1926; Taberly 1954b) have attributed the following characters to cryptoniscids of the family Dajidae: presence of oral sucker, article 1 of antenna 1 with long posteriorly directed projection, antenna 2 of nine articles, pereopod 1 short, with robust hooklike dactyl, pereopod 7 longer than preceeding ones and with rows of spines on palm of article 6, pleopods biramous, rami of uropods equal or subequal in length; if subequal, the endopodites are longer than the expodites.

The cryptoniscid of *H. alaskensis* lacks an oral sucker and a posteriorly directed projection on article 1 of antenna 1. In this respect it is similar to the cryptoniscid stage of *Zonophryxus quinquedens* Barnard (1913), but differs markedly in the structure of the uropods, the outer rami of the uropods of *Z. quinquedens* being reduced to small papilli-like structures. In addition, pereopod 1 of *H. alaskensis* is not markedly different morphologically from pereopods 2–6. In other respects the cryptoniscid of *H. alaskensis* conforms with the standard dajid characters listed above.

Nielsen and Strömberg (1973) considered the following to be important characters in distinguishing the species of cryptoniscids belonging to the superfamily Cryptoniscida: size and general body form, presence or absence of eyes, setation and dentation of articles 1 and 2 of antenna 1, length of antenna 2, orientation of buccal cone, number of teeth on coxal plates, appearance of pereopods, number of setae on pleopods, structure and setation of uropods, and shape of the pleotelson. These will probably also prove to be important characters for distinguishing the cryptoniscids of closely related members of the Dajidae.

The swimming behavior of the epicarid and cryptoniscid stage larvae, as observed in a petri dish, is distinctive. The epicarid stage swims a short distance in a wandering pattern, then stops and waves its pereopods, opening and closing the chelae, before swimming on. The cryptoniscids, on the other hand, dart in a single direction for a greater distance, with less of a tendency to wander.

The sex of individual *H. alaskensis* cannot be determined morphologically until metamorphosis from the cryptoniscid to the juvenile stage, after which females can easily be distinguished by their loss of pereopod 7. This is consistent with earlier authors (Reverberi and Pitotti 1942; Reverberi 1947; Reinhard 1949), who found that some bopyrids are sexually indetermined, becoming female if they attach to the host or male if they attach to the female. The position of attachment of the cryptoniscid to the host body wall over the branchiopericardial sinus and the eventual migration of the juvenile to the top of the carapace over the pericardium suggests that *H. alaskensis* feeds on decapod blood.

After the juvenile migrates to the top of the carapace, it becomes essentially immotile, losing all vestiges of body segmentation and suffering considerable reduction in pereopod articulation. It must, therefore, complete its metamorphosis, breed, and reproduce before the host molts. To avoid premature loss, the parasite may have some mechanism for inhibiting the molt.

## Acknowledgments

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# Influence of Substrate Composition and Suspended Sediment on Insect Predation by the Torrent Sculpin, *Cottus rhotheus*[1]

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BRUSVEN, M. A., AND S. T. ROSE. 1981. Influence of substrate composition and suspended sediment on insect predation by the torrent sculpin, *Cottus rhotheus*. Can. J. Fish. Aquat. Sci. 38: 1444–1448.

Studies conducted in laboratory streams showed that torrent sculpins (*Cottus rhotheus*) congregated in regions of limited cover when exposed to a sand substrate. When cobbles were added, their distribution was more uniform. Various combinations of sand, pebbles, and cobbles significantly influenced sculpin predation on the stonefly *Hesperoperla pacifica*, the mayfly *Ephemerella grandis*, and the caddisfly *Rhyacophila vaccua*. During 24-h tests, each species experienced 95–100% predation when tested on sand substrates. When different combinations of cobbles and pebbles were added, predation was appreciably reduced. Sculpin predation on *H. pacifica*, *Hydropsyche* sp., and *Heptagenia solitaria* was influenced more by substrate than suspended sediments when substrate and suspended sediment (0–1250 $mg \cdot L^{-1}$) were tested interactively. Predator avoidance tactics differed among prey species as changes were made in the physical characteristics of the environment.

*Key words*: predator–prey, sculpin, insects, suspended sediment, substrate, turbidity, behavior

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Des études en eau courante menées en laboratoire démontrent que les chabots de torrent (*Cottus rhotheus*) se rassemblent dans des régions avec abri limité lorsque exposés à un substrat de sable. Quand des moellons sont ajoutés au substrat, leur distribution est plus uniforme. Diverses combinaisons de sable, cailloux et moellons influencent de façon significative la prédation des chabots sur les perles *Hesperoperla pacifica*, les éphémères *Ephemerella grandis* et les phryganes *Rhyacophila vaccua*. Chaque espèce, lorsque testée sur des substrats de sable, subit une prédation de 95 à 100%. Quand un substrat et des sédiments en suspension (0–1250 $mg \cdot L^{-1}$) sont testés interactivement, le substrat exerce une plus grande influence que les sédiments en suspension sur la prédation des chabots sur *H. pacifica*, *Hydropsyche* sp. et *Heptagenia solitaria*. Quand les caractéristiques physiques de l'environnement sont modifiées, les tactiques d'évitement du prédateur par les espèces proies diffèrent d'une espèce à l'autre.

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THE behavior, distribution, and abundance of insects and fish are directly or indirectly influenced by the physical characteristics of a stream. Predator–prey relationships of these organisms contribute to an orderly transfer of energy, thus leading to community stability (Odum 1971). As watersheds are altered, streams and rivers undergo physical and chemical changes which alter biological processes and result in ecosystem instability. Suspended sediments and substrate characteristics are important factors associated with watershed alterations.

Cummins (1966) indicated that physical characteristics of the streambed are of great biological significance. Large deposits of sand and silt alter the characteristics of streams and cause changes in the benthic insect community (Cordone and

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Kelley 1961; Cummins and Lauff 1969; Brusven and Prather 1974; Rosenberg and Wiens 1975; Luedtke and Brusven 1976; McClelland and Brusven 1980). The effects of suspended sediments on benthic invertebrates has been measured primarily as a drift response, with increased suspended sediment concentration causing increased drift (Pearson and Franklin 1968; White and Gammon 1977).

Griffin (1938) noted that juvenile cutthroat trout (*Salmo clarki*) and chinook salmon (*Oncorphynchus tshawytscha*) continued to feed at suspended sediment concentrations >500 mg·L$^{-1}$. However, Olson et al. (1973) reported rainbow trout (*Salmo gairdneri*) feeding decreased sharply when turbidity exceeded 70 Jackson turbidity units (JTU), which for most sediment sources is considerably less than 500 mg·L$^{-1}$ by weight.

Because sculpins, common nongame fish in many streams and rivers in North America, are potential competitors in the food chain of game species, we conducted a series of laboratory experiments to study the poorly known predator–prey relationships of sculpins and insects under different substrate and suspended sediment conditions. The effects of these factors would help explain the feeding behavior of sculpins and provide a basis for making inferences about other vertebrate predators.

*Materials* — Plexiglas oval streams (1.6 m × 0.51 m × 0.23 m), similar to those described by Brusven (1973), were used to investigate predator–prey interactions. Each stream had three habitat types: (1) end zone, (2) turbulent zone, and (3) non-turbulent zone (Fig. 1). Black Visqueen plastic was placed around the sides of the streams to minimize visual disturbance to the fish. A self-contained recirculation system was used. Stream discharge was maintained at 30.8 L/min at 10 cm in depth with a continuously run centrifugal pump. Temperature was held at a relatively constant level (11 ± 1°C) with a thermostatically controlled compressor unit attached to a stainless steel cooling coil. Four-tube, 1.2-m-fluorescent-light fixtures were suspended 0.6 m above the streams and set for a 12-h dark–12-h light cycle with electronic timers.

A commercially screened clay–sand mixture (particle size <0.15 mm) was used to give desired suspended sediment levels in the stream. The material was introduced in the form of a slurry. Suspended sediment concentrations (mg·L$^{-1}$) were determined by filtration, drying, and weighing of sediment. The mg·L$^{-1}$ concentrations were scaled to JTUs as measured with a Hach surface scatter turbidimeter and continuously recorded with an Esterline Angus minigraph strip-chart recorder. A solenoid was electronically interfaced with the circuitry of the Hach turbidimeter so that a small amount of the slurry was automatically added when the low-sensitivity circuit was activated. Most of the coarser particles settled out within 6 h, but these did not appreciably alter the character of the substrate during a 24-h test.

Torrent sculpins (*Cottus rhotheus*) were obtained in the field by electrofishing. They were acclimated in holding streams similar to test streams for a minimum of 78 h prior to testing. Sculpins ranging from 6.6 to 9.0 cm long were used. Three groups of eight were segregated in the holding stream. Test groups were starved for 48 h prior to testing. Patten (1971) reported that a 48-h starvation period for the torrent sculpin increased the rate of predation by seven times over a 24-h period. Live insects (species similar to test prey) were fed to the sculpins when periods of longer than 48 h occurred between tests. During the study, several groups of sculpins were obtained from the field because of occasional fish mortality in holding streams and unavailability of desired prey species during all times of the year. We observed no evidence of acute toxicity from suspended sediments to either sculpins or insects during the 24-h tests.

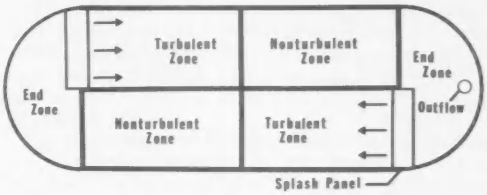


FIG. 1. Configuration of an artificial stream with habitat zones indicated.

Insects used as prey were collected in the field by a kick screen. The mayflies *Ephemerella grandis* and *Heptagenia solitaria*, the stonefly *Hesperoperla pacifica*, and the caddisflies *Rhyacophila vaccua* and *Hydropsyche* sp. were used.

*Methods — Predation vs. substrate cover* — Insect predation by *C. rhotheus* was tested in laboratory streams on different substrate types. Rates of predation were determined for five substrate conditions: (1) sand (0.5–1.5 mm), (2) sand with cobbles (10.0–11.5 cm) unembedded in sand, (3) sand with cobbles half-embedded, (4) sand with pebbles (1.2–2.5 cm), and (5) pebble-covered sand with embedded cobbles. When cobbles were used, six having similar color, surface texture, and size were evenly spaced in each of the four rectangular quadrants of the stream.

Eight sculpins were placed in the test stream and allowed to acclimate for 15 min. Thirty insects of a given species (*E. grandis*, *H. pacifica*, and *R. vaccua*) were evenly introduced among the test sections of the stream. Each test was run for 24 h and replicated three times using different fish and insects. At the end of a 24-h test, divider screens were inserted between the test quandrants and between the test quadrants and end zones; the density and distribution of predators and prey were recorded.

*Predation vs. substrate cover and suspended sediment concentration* — The interactive effects of substrate and suspended sediments on sculpin predation on *Hydropsyche* sp., *H. solitaria*, and *R. pacifica* were studied at three levels of turbidity (clear: 4–6 JTU; moderate:100–150 JTU ≃ 310 mg·L$^{-1}$; and high:300–350 JTU ≃ 1250 mg·L$^{-1}$) and two substrate conditions (sand, sand with embedded cobbles). Eight sculpins and 30 insects of a given species were introduced into the stream. Tests were run for 24 h and replicated three times. Each test used a different group of sculpins and insects. At the end of a test, screened dividers were placed between the quandrants of the stream and between the end zones of the quadrants; the water level was lowered, and location and numbers of insects and fish were recorded.

We tested the interactive effects of substrate and suspended sediment on predation using a Kruskal–Wallis test at the 5%

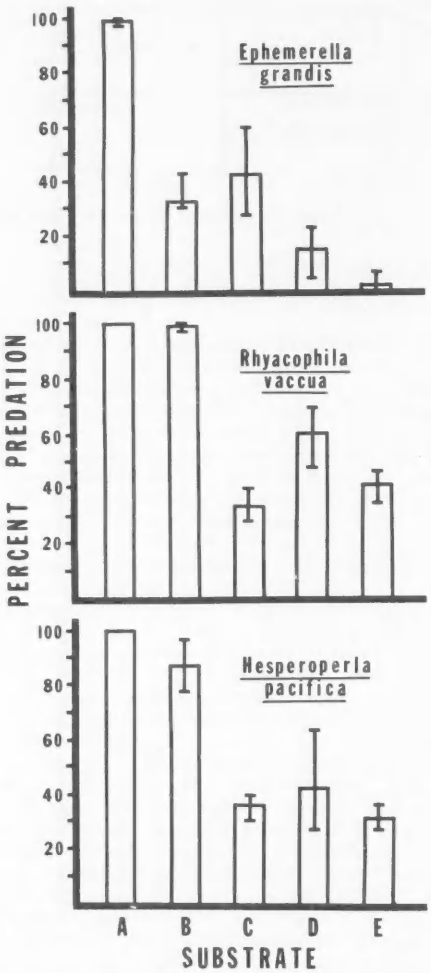


FIG. 2. Percent predation by *Cottus rhotheus* on three species of insects subjected to five substrate conditions. A = sand, B = sand with half-embedded cobbles, C = sand with unembedded cobbles, D = sand with pebbles, and E = pebble-covered sand with unembedded cobbles. Vertical lines represent range of three replications.

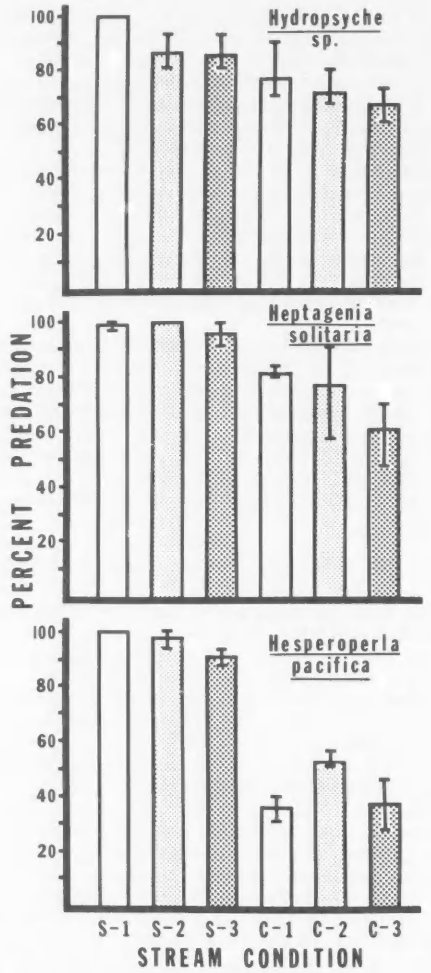


FIG. 3. Percent predation by *Cottus rhotheus* on three species of insects subjected to two substrate conditions (S = sand, C = unembedded cobbles on sand) and three suspended sediment concentrations (1 = clear, 0 mg·L$^{-1}$; 2 = moderate turbidity 310 mg·L$^{-1}$; 3 = high turbidity, 1250 mg·L$^{-1}$). Vertical lines represent range of three replications.

level. To elucidate the individual significance of suspended sediment and substrate on insect predation, we used a Mann–Whitney test which provided a nonparametric protected least significance comparison; because of the low number of replications involved in pairwise comparisons, we used a 10% level for significance. We tested the distribution of fish among stream regions at the 5% level with a chi-square contingency test.

*Results* — Five substrate conditions caused different levels of predation by *C. rhotheus* on three species of insects (Fig. 2). A Kruskal–Wallis nonparametric one-way ANOVA showed that, for each prey species, the substrate had a significant effect on percent predation. Not all substrate treatments for a species were different when compared pairwise, however. In most cases predation on sand clearly differed from most of the other treatments. Predation on sand and sand with half-embedded cobbles was nearly equal for *R. vaccua* and *H. pacifica* but not for *E. grandis*. Individuals of *E. grandis* most successfully escaped predation (90% escaped on a substrate of pebbles on sand with unembedded cobbles).

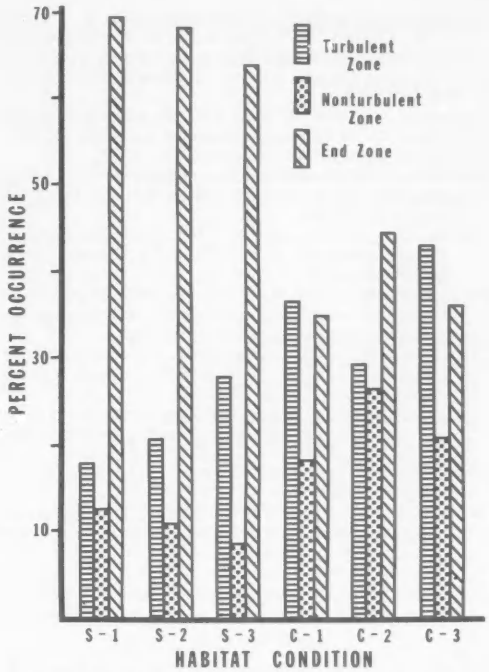


FIG. 4. Relative occurrence of *Cottus rhotheus* from three stream regions having three suspended sediment concentrations and two substrate conditions: S = sand, C = cobble on sand. 1 = clear, 0 mg·L$^{-1}$; 2 = moderate turbidity, 310 mg·L$^{-1}$; 3 = high turbidity, 1250 mg·L$^{-1}$.

Three suspended sediment concentrations and two substrate conditions caused profoundly different sculpin predation on three species of insects (Fig. 3). The interactive effects of substrate and suspended sediment on predation were significant. When testing the influence of suspended sediment levels alone on predation on the three species of insects, we found significant differences in only one of nine comparisons (i.e. *Hydropsyche* sp. clear vs. high turbidity). However, when comparing the effects of substrate composition alone, significant differences were demonstrated for all three prey species. The least difference in predation between substrates was noted for *Hydropsyche* sp. For the conditions tested, substrate condition rather than suspended sediment had the greatest influence on depressing predation.

Fish distribution within the stream was highly significant ($P < 0.01$) for stream region (Fig. 4). On a sand substrate without cobbles, the end zones were preferred habitats over the linear turbulent and nonturbulent regions. When cobbles were placed in the latter two regions, sculpin distribution was more uniform among the three habitat types, with the nonturbulent zone being least utilized.

*Discussion* — Studies by Brusven and Prather (1974) showed that selected species of mayflies, stoneflies, and caddisflies preferred substrates having cobble-sized rocks rather than sand and pebbles, and that cobbles were increasingly more preferred as the sediment particles under or around cobbles decreased in size. We believe the time spent by prey on or under rocks and pebbles of the streambed, cryptic coloration, and diel behavior strongly influence the vulnerability of insects to predation.

Our present study shows that the distribution of *C. rhotheus* is also greatly influenced by substrate composition. Fish congregated in the end zones, especially under the splash panels (the only available cover) when a uniform sand substrate was present. When cobbles were placed in the turbulent and nonturbulent quadrants, the fish were more uniformly distributed among the three habitats. The preference of sculpin for cobbles agrees with our observations in the field, and those of Bailey (1962) and Bond (1963). Bond (1963) and Patten (1971) reported *C. rhotheus* feeding in late afternoon, morning or night; we observed them feeding during daylight hours in laboratory streams. Most of the feeding occurred when insects passed within 15 cm of the sculpins. We observed little evidence of the fish moving from one region of the stream to another in quest of prey even though prey were actively moving. For the most part, the fish resided on the bottom of the streambed, often partially covered with fine sand.

Various combinations of sand, pebbles, and cobbles greatly influenced sculpin predation on *H. pacifica*, *E. grandis*, and *R. vaccua*. During all tests involving sand substrate, 95–100% predation occurred on all three species. When cobbles and pebbles were added, considerable reduction in predation occurred.

The predation observed can be explained by differences in prey behavior. *Ephemerella grandis* is an inactive, slow-moving insect that typically lives under, on the sides of, or in cracks and depressions of rocks and debris. This insect is cryptic and not easily detected by a sight-feeding predator when stationary or inactive. Further protection resulted when cobbles were present; predation with cobbles was less than half that on sand without cobbles.

*Hesperoperla pacifica* and *R. vaccua* differed from *E. grandis* in their vulnerability to predation when subjected to unembedded and half-embedded cobbles. Both species are more active than *E. grandis* and prefer the undersurface of cobbles and pebbles. When cobbles were unembedded in sand, predation was ~35%, but when cobbles were half-embedded, predation was >90%. The undersurfaces of cobbles become less available to these insects as the cobbles become more embedded and the substrate particles around them become smaller. Differential palatability was not a factor among these three species because nearly 100% predation occurred when tested with sand. The same or similar insects have been reported fed upon by sculpin (Bailey 1952; Northcote 1954; Antonelli et al. 1972; Novak and Estes 1974).

Our studies showed that the substrate influenced predation by *C. rhoteus* on *Hydropsyche* sp., *H. solitaria*, and *H. pacifica* more than suspended sediments at a concentration of 1250 mg·L$^{-1}$. Only the net-spinning caddisfly *Hydropsyche* sp. and the mayfly *H. solitaria* were preyed upon less in highly turbid water (1250 mg·L$^{-1}$) than clear water when tested on a substrate of unembedded cobbles on sand. All three prey species were relatively active and potenially vulnerable to predation during the initial hours of a test. *Hydro-*

*psyche* sp. began net construction shortly after being introduced into the stream. The few individuals escaping predation during a 24-h test had partially constructed nets on the walls of the stream when cobbles were absent or on cobbles when they were present. We suspect that predation on this or similar species would be reduced once nets were fully constructed because the larvae would be less conspicuous to a sight-feeding predator. Highly secretive and negatively phototactic behavior of *H. pacifica* undoubtedly contributed to the lower overall predation when cobbles were present as cover. The results of our experiment show that predator avoidance tactics differ among species and that differences in behavior and the physical characteristics of environment contribute to this variation.

Peckarsky (1980) reported highly variable predator–prey interactions between stoneflies and mayflies. In addition to visual and tactile stimuli, she proposed that contact and non-contact chemoreception are possible mechanisms involved in predator avoidance. One can only speculate that if indeed chemoreception exists between predator and prey insects, then it may also be operative in fish–insect interactions.

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# Distribution tissulaire du cadmium chez *Meganyctiphanes norvegica* (Euphausiacée) : état naturel et accumulation expérimentale de formes solubles

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Chez *Meganyctiphanes norvegica* prélevé dans son milieu naturel, 57 % de son contenu en cadmium est associé à des composés solubles constitués principalement de protéines (PM ⩾ 25 000). Des expériences de contamination ont permis l'induction d'une protéine riche en cadmium d'un poids moléculaire d'environ 21 000. Toutefois, une portion importante du cadmium est fixée sur l'exosquelette. Cette fixation du métal sur les parties chitineuses et son éventuelle élimination par les exuvies peuvent constituer un mode de défense particulier aux crustacés.

*Mots clefs :* euphausiacée, *Meganyctiphanes*, cadmium, métallothionéine, métaux en trace

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In the tissues of *Meganyctiphanes norvegica* collected from their natural environment, 57% of body cadmium was in soluble compounds, principally bound with proteins (mol wt ⩾ 25 000). A cadmium-binding protein, with a molecular weight of ~ 21 000, was induced during laboratory contamination experiments. However, a large portion of cadmium is adsorbed onto exoskeleton. The association of the metal with chitin and its removal by molting may constitute a defence process particular to crustaceans.

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PLUSIEURS auteurs ont montré l'importance des processus biologiques dans le transport des éléments en trace de l'eau vers les sédiments marins (Chester et Aston 1976; Wallace et al. 1977). Dans le cas du cadmium, le phytoplancton semble jouer le plus grand rôle dans les eaux de surface (Boyle et al. 1976; Bruland et al. 1978) alors que le zooplancton serait responsable par la production de pelotes fécales et d'exuvies d'une grande partie du transport vertical du cadmium vers le fond (Fowler 1977). La clarification du rôle du zooplancton dans le cycle du cadmium dans l'océan requiert la connaissance du métabolisme de ce métal chez ces organismes.

Le métabolisme du cadmium chez les organismes marins a été peu étudié. Des travaux, effectués chez des mollusques et des vertébrés marins, ont montré l'association du cadmium aux protéines solubles et ont mis en évidence l'induction par ce métal de protéines riches en cadmium, les métallothionéines ayant probablement un rôle de détoxification (Olafson et Thompson 1974; Noël-Lambot 1976). Chez les crustacés, des cinétiques d'accumulation ont d'abord été établies (v.g. Benayoun et al. 1974). Récemment les travaux d'Olafson et al. (1979 a, b), de Jennings et al. (1979) et de Rainbow et Scott (1979) rapportent l'existence de métallothionéines chez certaines espèces de crabe et de crevette.

Afin d'apporter quelques éléments supplémentaires au métabolisme du cadmium chez le zooplancton, nous avons examiné ici la distribution du cadmium au niveau tissulaire et protéinique chez une euphausiacée assez largement distribuée (*Meganyctiphanes norvegica*). Cette étude a été effectuée chez des animaux à l'état naturel et après contamination par des formes solubles du cadmium.

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*Matériel et méthodes* — Les euphausiacées, *M. norvegica*, ont été recueillies au filet à plancton, la nuit, aux environs de 40 m, durant l'été 1977. Les animaux adultes de 3 à 4 cm de long ont été recueillis à deux stations dans l'estuaire maritime du Saint-Laurent : au large de Rivière-la-Madeleine (49°25′N, 65°20′O) et au large de Rimouski (48°37′N, 68°31′O). Les températures et salinités lors des récoltes variaient respectivement de 3 à 5°C et de 28 à 32 ‰.

Les euphausiacées destinées aux analyses de la distribution du cadmium à l'état naturel ont été congelées au fréon immédiatement après leur pêche et conservées à −80°C jusqu'au moment de leur analyse.

Les expériences de contamination ont été effectuées dans des aquariums de verre d'une capacité de 2,3 L et contenant 2 L d'une eau de mer de salinité 28 ‰ filtrée sur membrane Millipore de 0,4 μm de porosité. La température de l'eau a été maintenue à 4 ± 1°C pendant toute la durée de l'expérience. La photopériode était de 12 h par 24 heures. Lors des expériences, quatre euphausiacées ont été placées dans chaque aquarium et maintenues à jeun pendant les 7 jours d'expérience. Un milligramme (1 mg) de cadmium sous forme de $CdCl_2$ a été ajouté à chaque aquarium de façon à obtenir une concentration de 500 μg $Cd \cdot L^{-1}$. La solution d'incubation a été renouvelée chaque 24 h afin de conserver les animaux dans un milieu suffisamment oxygéné et de minimiser les variations de concentration en cadmium durant l'expérience. Après 7 jours d'incubation en milieu contaminé, le taux de mortalité des euphausiacées atteint 76 % alors que chez les animaux témoins il est resté inférieur à 5 %. Le choix d'une concentration létale de cadmium a été dicté par l'absence de modification observable de la distribution du cadmium protéinique à des concentrations inférieures. Les analyses ont, toutefois, été effectuées chez les animaux ayant survécu.

Les euphausiacées ont été sacrifiées par congélation au fréon et conservées à −80°C jusqu'à leur analyse. Aux 1er, 3e et 7e jours d'incubation, quatre animaux ont été utilisés pour étudier la distribution tissulaire du cadmium; au 7e jour, trois lots de deux euphausiacées ont été utilisés pour l'établissement des chromatogrammes de protéines.

La séparation des composés solubles est basée sur la méthode de Shaikh et Lucis (1971). En bref, les euphausiacées sont homogénéisées au Virtis dans une solution de tampon (Tris−HCl 0,001 mol $\cdot L^{-1}$, pH 8,6 contenant 0,02 % de $NaN_3$ et 0,25 mol $\cdot L^{-1}$ de saccharose). Le broyat centrifugé à 8000 × *g* pendant 25 min afin d'éliminer les débris des membranes. Le surnageant est soumis à une ultracentrifugation à 105 000 × *g* pendant 90 min destinée à ne conserver dans le surnageant que les composés cytoplasmiques. Ce surnageant est alors lyophylisé et repris dans 0,5 mL d'eau distillée juste avant son application sur la colonne chromatographique (Pharmacia, K16-100; gel Sephadex G-75). L'échantillon puis 2 mL d'une solution de saccharose à 10 % sont appliqués sur le gel. L'éluant (tampon Tris−HCl 0,001 mol $\cdot L^{-1}$ contenant 0,02 % de $NaN_3$, pH 8,6), après passage ascendant dans la colonne est recueilli par fractions de 3,8 mL par un collecteur de fractions (Gilson, MFK). Les protéines sont mesurées à 250 à 280 nm (Noël-Lambot 1976) avec un spectrophotomètre Perkin−Elmer (modèle 575). Un pourcent d'acide nitrique est alors ajouté aux fractions afin de libérer le cadmium des protéines (George et al. 1979) et favoriser ainsi le dosage du cadmium effectué par spectrophotométrie d'absorption atomique sans flamme (Perkin−Elmer 460, HGA-2100).

L'analyse du cadmium dans les tissus des euphausiacées est effectuée aussi par spectrophotométrie d'absorption atomique sans flamme sur le méneralisat obtenu par oxydation nitrique des tissus dans des réacteurs en Teflon selon la technique décrite par Cossa et al. (1980).

TABLEAU 1. Distribution du cadmium chez *M. norvegica* en milieu naturel renfermant (50 ng $Cd \cdot L^{-1}$).

| Fraction | % du poids sec total | % du cadmium total |
|---|---|---|
| *Tissus* | | |
| Viscères (+ hémolymphe) | 22,7 | 35,5 |
| Exosquelette | 22,1 | 26,2 |
| Muscle | 50,6 | 33,5 |
| Branchies (+ yeux) | 4,4 | 4,8 |
| *Composés solubles* | | |
| Total | — | 57 |
| (PM ⩾ 25 000) | — | (93)[a] |
| (10 000 ⩽ PM ⩽ 25 000) | — | (Traces) |
| (PM ⩽ 5 000) | — | (7)[a] |

[a]Pourcentage par rapport au cadmium associé aux composés solubles.

*Résultats et discussion — Distribution du cadmium chez M. norvegica en milieu naturel* — La concentration moyenne en cadmium (± l'intervalle de confiance à 95 %) mesurée chez cinq individus de *M. norvegica* de l'estuaire maritime du Saint-Laurent est de 0,34 ± 0,10 μg $\cdot g^{-1}$ poids sec. Cette valeur se situe parmi les plus faibles concentrations en cadmium mesurées dans différentes régions chez des organismes zooplanctoniques (Davies 1978). De plus, si l'on considère la concentration moyenne en cadmium dans l'eau de mer mesurée dans la même région (50 ng $\cdot L^{-1}$, Cossa et al. 1981) et si un rapport poids/humide poids sec de 4,7 pour l'animal est utilisé, un facteur de concentration du cadmium de 1447 peut être calculé entre *M. norvegica* et son milieu. Ce chiffre est très proche de la valeur de 1500 obtenue pour le même métal chez la même espèce en mer Méditerranée (Benayoun et al. 1974).

Près de 70 % du cadmium sont répartis en quantités équivalentes entre la masse viscérale et le muscle (tableau 1). Les 30 % restant sont principalement associés à l'exosquelette et aux branchies (tableau 1). Les résultats montrent aussi une très forte participation des composés solubles dans la fixation du cadmium (tableau 1). Le cadmium lié aux composés solubles représente 83 % de ce métal lié aux viscères, à l'hémolymphe et au muscle.

Les chromatogrammes des composés solubles et du cadmium lié (fig. 1) montrent une répartition en deux fractions principales. Ce type de chromatogrammes est commun à de nombreuses espèces animales (v.g. rat, cheval, phoque, anguille, sébaste). La première fraction de poids moléculaire ⩾25 000 comprend les métallo-enzymes, l'autre de poids moléculaire ⩽5000, d'après Brown et al. (1977), doit être

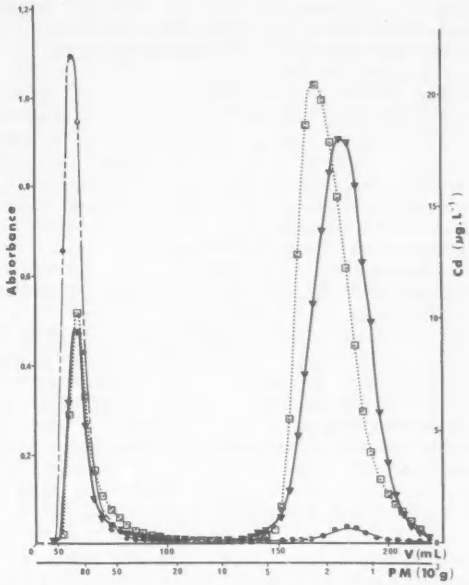


FIG. 1. Chromatogramme des protéines et du cadmium associé chez *M. norvegica* en milieu naturel renfermant 50 ng Cd · $L^{-1}$ séparés sur colonne de gel (Sephadex G-75). Cercles pleins, concentration en Cd; triangles, absorbance à 250 nm; carrés, absorbance à 280 nm. En abscisse figurent le volume d'élution (*V*) et le poids moléculaire correspondant (PM) calculé à partir d'une calibration effectuée avec cinq standards protéiques (albumine, chymotrypsinogène, myoglobine, RNase A et insuline).

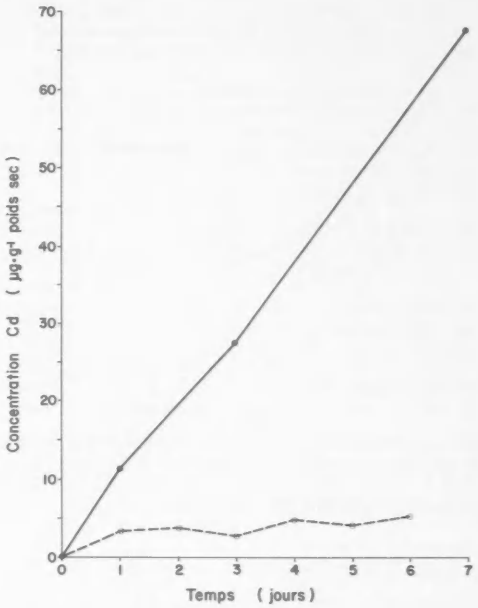


FIG. 2. Accumulation du cadmium chez *M. norvegica* au cours de l'expérience de contamination à 500 µg Cd · $L^{-1}$. Cercles pleins, animal entier; cercles vides, composés solubles.

composée d'un mélange de polypeptides, de fragments d'acides nucléiques et de molécules biologiques de petite taille. Quatre-vingt-treize pourcent (93 %) du cadmium sont associés avec la première fraction, c'est-à-dire avec des protéines de poids moléculaire supérieur à 25 000. Toutefois, dans la fraction de petit poids moléculaire, la position du maximum de cadmium coïncide avec celle du pic à 250 nm (fig. 1). L'absorbance à 250 nm traduisant selon Wisniewska–Knypl et al. (1971) le lien cadmium-mercaptan, ces composés sont probablement de polypeptides riches en acides aminés à groupement -SH. Ainsi le cadmium associé aux composés solubles, qui représente 57 % de ce métal chez *M. norvegica*, est dans sa quasi-totalité lié à du matériel protéinique. Vingt-six pourcent (26 %) du cadmium étant fixé aux parties, chitineuses de l'animal (tableau 1), il s'en suit qu'une faible portion du métal (17 %) seulement peut se trouver combinée à des structures membranaires, aux membranes plasmatiques, ou à des granules inorganiques tels que décrits par Coombs et Georges (1978).

*Distribution du cadmium chez M. norvegica au cours des expériences de contamination* — L'évolution de la concentration en cadmium dans les différents tissus de *M. norvegica* a été suivie pendant 7 jours d'incubation des animaux dans le milieu artificiellement contaminé. La figure 2 illustre l'accumulation continue chez l'animal entier, qui n'atteint pas un état d'équilibre avec le milieu après 7 jours d'exposition, et la saturation en cadmium des composés solubles après les premières 24 h. Au début de l'expérience on assiste à une augmentation très forte de la proportion de cadmium associée aux branchies (fig. 3). Des observations semblables ont été faites chez le crabe *Carcinus maenas* par Jennings et Rainbow (1979). Ceci résulte vraisemblablement du fait que les branchies constituent une voie importante d'entrée d'ions et un site de fixation privilégiée du cadmium dissous en raison du flux rapide d'eau provoqué par les organes filtreurs (Mauchline et Fisher 1969). La chute brutale de la quantité relative de cadmium liée aux composés solubles, qui passe de 57 % à 28, 20 et 10 % après 1, 2 et 3 jours d'incubation, traduit la saturation rapide des protéines (fig. 2) et l'accumulation par les parties chitineuses (fig. 3) dans les premiers jours d'expérience. Par la suite, l'enrichissement progressif du métal dans le muscle, comparativement aux branchies (fig. 3), montre la pénétration du cadmium dans l'organisme.

Le chromatogramme, obtenu après 7 jours d'incubation des animaux, montre un nouveau pic correspondant à une protéine d'un poids moléculaire de 21 000 (fig. 4). Cette protéine, détectable uniquement par son contenu en métal, s'apparente par son poids moléculaire à la famille des métallothionéines. Deux travaux récents démontrent en effet l'existence de différentes métallothionéines chez les crustacés : Olafson et al. (1979b) ont déterminé un poids moléculaire de 9500 chez la crevette *Acetes sibogae* et Jennings et al. (1979) font état de

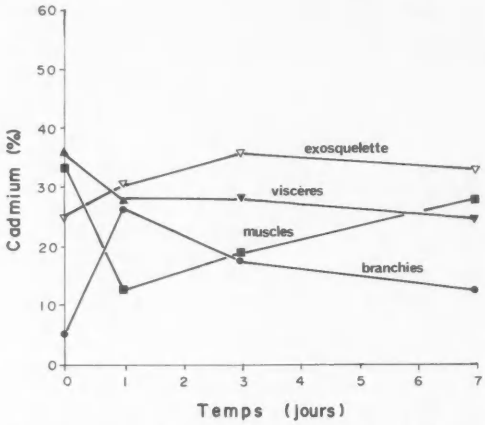


FIG. 3. Évolution de la distribution du cadmium dans les tissus de *M. norvegica* au cours de l'expérience de contamination à 500 μg $Cd \cdot L^{-1}$. Cercles pleins, branchies; carrés pleins, muscle; triangles pleins, viscères; triangles vides, exosquelette.

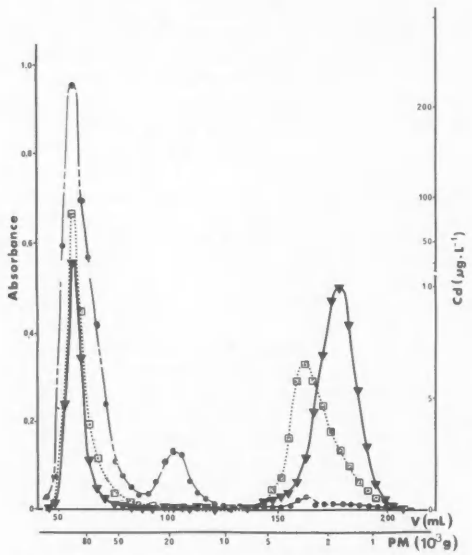


FIG. 4. Chromatogramme des protéines et du cadmium associé chez *M. norvegica* après 7 jours d'incubation dans un milieu contaminé par 500 μ g $Cd \cdot L^{-1}$ séparés sur colonne de gel (Sephadex G-75). Voir légende de la figure 1.

l'existence de deux protéines riches en cadmium chez le crabe (*C. maenas*) ayant des poids moléculaires respectifs de 11 500 et 27 000. La protéine riche en cadmium mise en évidence ici chez *M. norvegica* pourrait, comme le suggèrent Jennings et al. (1979) pour la métallothionéine de 27 000 chez *C. maenas*, constituer un dimère d'une protéine de structure proche de la plupart des métallothionéines mises en évidence dans le règne animal (v.g. Kagi et Vallee 1960, 1961; Pulido et al. 1966; Wisniewska–Knypl et al. 1971; Olafson et Thompson 1974; Noël-Lambot 1976; Noël-Lambot et al. 1978). La mise en évidence de cette protéine riche en cadmium chez *M. norvegica* confirme la diversité structurelle des métallothionéines chez les animaux marins. Cette diversité dans le même phyllum est renforcée par la mise en évidence dans notre laboratoire, au cours d'expériences semblables à celles effectuées sur *M. norvegica*, d'une protéine de poids moléculaire de 11 200 chez une autre espèce d'euphausiacée de l'estuaire du Saint-Laurent (*Tyssanoessa inermis*).

Cependant chez les crustacés l'induction de la synthèse des métallothionéines nécessite des concentrations en cadmium plus élevées que chez les autres organismes (500 μg $Cd \cdot L^{-1}$ dans nos travaux et ceux de Jennings et al. (1979) et de Olafson et al. (1979 a, b)). Des contaminations expérimentales conduites à 10 μg $Cd \cdot L^{-1}$ n'ont en effet pas permis de mettre en évidence un quelconque changement dans l'allure des chromatogrammes chez *M. norvegica*. C'est en fait vers la fraction de haut poids moléculaire que se dirige la plus grande partie (97 %, fig. 4) du cadmium lié par les protéines solubles. Brown et Parsons (1978) ont observé une situation similaire au cours d'expériences sur du zooplancton contaminé par du mercure : une limitation de la production de métallothionéines et une augmentation subséquente de la quantité de mercure sur la fraction des métallo-enzymes les ont conduits à suggérer un débordement du métal d'une fraction à l'autre.

En résumé, nos résultats montrent, chez *M. norvegica*, l'affinité du cadmium pour le matériel protéinique et la présence d'un mécanisme de détoxification du cadmium par la synthèse d'une métalloprotéine riche en cadmium s'apparentant aux métallo-thionéines. De plus, ils confirment la diversité structurelle des métallothionéines à travers l'arbre phylogénique. Toutefois, chez les euphausiacées, comme chez d'autres crustacés, une proportion importante du cadmium est adsorbée sur l'exosquelette. Cette fixation du métal sur les parties chitineuses et son élimination éventuelle par les exuvies peut constituer un mode de défense supplémentaire par rapport aux autres animaux et expliquer la capacité limitée de synthèse de métallothionéines observée chez les crustacés.

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RAINBOW, P. S. ET A. G. SCOTT. 1979. Two heavy metal-binding proteins in the midgut gland of the crab *Carcinus maenas*. Mar. Biol. 55: 143–150.

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## Books received/Livres reçus[1]

*June–July/juin–juillet 1981*

**Man, Fishes, and the Amazon.** *Nigel J. H. Smith. Columbia University Press, New York, NY 10025. 1981. x + 180 p. $22.50 U.S.* An original history of the fishermen and fish of the Amazon basin with major emphasis on the fisheries per se.

**Freshwater Fishes of New York State.** *Robert G. Werner. 1980. Syracuse University Press, Syracuse, New York 13210. vi + 186 p. $20.00 (cloth), $11.95 (paper) U.S.* This is a field guide geared to the amateur naturalist and fisherman. Illustrated identification keys as well as life history and distribution data for the 68 common species are included.

**Law of the Sea: Problems of Conflict and Management of Fisheries in Southeast Asia.** *Edited by Francis T. Christy Jr. ICLARM Conference Proceedings No. 2. 1980. xxi + 68 p.* Proceedings of the ICLARM/ISEAS Workshop held in Manila, Philippines, November 1978. Fisheries for scads, mackerels, and tuna and regulations concerning foreign and domestic fisheries are discussed.

**Integrated Agriculture-Aquaculture Farming Systems.** *Edited by Roger S. V. Pullin and Ziad H. Shedadeh. ICLARM Conference Proceedings 4. 1980. vii + 25 p.* Proceedings of the ICLARM–SEARCA Conference held in Manila, Philippines, August 1979 and comprising 10 review papers and 13 case studies.

**Review of Breeding and Propagation Techniques for Grey Mullet, *Mugil cephalus* L.** *Edited by Colin E. Nash and Ziad H. Shehadeh. ICLARM Studies and Reviews 3. 1980. vii + 87 p.*

**Philippine Municipal Fisheries: a Review of Resources, Technology and Socioeconomics.** *Ian R. Smith, Miguel Y. Puzon and Carmen N. Vidal-Libunao. ICLARM Studies and Reviews 4. 1980. ix + 87 p.* Reviews the problems of small scale fisheries in an "open-access" situation.

**Food Potential of Aquatic Macrophytes.** *Peter Edwards. ICLARM Studies and Reviews 5. 1980. viii + 51 p.*

**Aquatic Toxicology.** *Elsevier/North Holland. Vol. 1, No. 1, April 1981. 83 p. Dfl 180.00 (U.S. $92.25) for a complete volume of six issues.* This new journal is a vehicle for original studies emphasizing mechanisms and assessment of toxicity in aquatic environments from the subcellular to ecosystem level. Also covered will be new techniques and procedures.

**Fish Diseases.** *Edited by W. Ahne. Proceedings in life sciences. Springer-Verlag. Berlin, Heidelberg, New York. 1980. x + 252 p. $49.80 U.S.* Contributions on viral and bacterial diseases from the Third COPRAQ (Cooperative Programme of Research on Aquaculture) - Session held at Munich, October 1979.

**Principles of Fishery Science.** *W. Harry Everhart and William D. Youngs. Cornell University Press, Ithaca, New York. 1981. Second edition. 349 p. $16.50 U.S.* A text to help students, professionals, and managers reach a thorough understanding of the problems of providing the optimum yield of fishery products. The treatment is interdisciplinary and a new appendix provides a review of the mathematics and statistics required for understanding population ecology.

**Journal of Crustacean Biology.** *The Crustacean Society, c/o R. B. Manning IZ-NHB-W323, Smithsonian Institution, Washington, D.C. 20560. 1981. Vol. 1, No. 1. 151 p. $25 U.S. in North America, $30 outside North America for a complete volume of four issues.* This quarterly will include results of research on any aspect of the biology of Crustacea.

**Oil Spill Cleanup and Protection Techniques for Shorelines and Marshlands.** *Edited by A. Breuel. Noyes Data Corporation, Park Ridge, New Jersey. Pollution Technology Review No. 78. 1981. xii + 404. $42 U.S.* Prepared from existing documents, this book provides information on assessments of contamination and guidelines for determining the most effective techniques for protection, cleanup, and restoration.

**The Chemical Biology of Fishes, Vol. 2.** *R. Malcolm Love. Academic Press Inc. (London) Ltd. London, England. 1980. xviii + 943 p. $120.00.*

[1]Persons interested in reviewing books in this list should contact the Book Review Editor, Canadian Journal of Fisheries and Aquatic Sciences, Scientific Information and Publications Branch, Department of Fisheries and Oceans, Ottawa, Canada K1A 0E6.

[1]Les personnes intéressées à examiner les livres énumérés dans cette liste sont priées de communiquer avec le Rédacteur des comptes rendus, Journal canadien des sciences halieutiques et aquatiques, Direction de l'information et des publications scientifiques, Ottawa, Canada K1A 0E6.

## COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Forward to the Stock Concept Symposium **C. M. Fetterolf Jr.**

Introduction to the Proceedings of the 1980 Stock Concept International Symposium (STOCS) **A. H. Berst and R. C. Simon**

Who's in charge here? **J. R. Harlan**

Where are we now? **K. H. Loftus**

Changing management of Great Lakes fish stocks **D. P. Borgeson**

A perspective on population genetics and salmon management **P. A. Larkin**

Stock definition as a necessary basis for cooperative management of Great Lakes fish resources **J. H. Kutkuhn**

The conundrum of the stock concept — are nature and nurture definable in fishery science? **H. E. Booke**

Behavioral stock-isolating mechanisms in Great Lakes fishes with special reference to homing and site imprinting **R. M. Horrall**

Stocks are chameleons: plasticity in gill rakers of coregonid fishes **C. C. Lindsey**

Use of allelic frequencies to describe population structure **F. W. Allendorf and S. R. Phelps**

The stock concept and the uncoupling of organismal and molecular evolution **J. W. Clayton**

The stock concept from the viewpoint of population genetics **Yu. P. Altukhov**

Origin and geography of the fish fauna of the Laurentian Great Lakes basin **R. M. Bailey and G. R. Smith**

Genetic perspectives of the identification and conservation of Scandinavian stocks of fish **N. Ryman and G. Ståhl**

Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness and current management **J. E. Thorpe and K. A. Mitchell**

Applications of the stock concept to fish populations in the USSR **Yu. P. Altukhov and E. A. Salmenkova**

Stock characteristics and catches of Atlantic salmon, *Salmo salar*, in Quebec, and Newfoundland and Labrador in relation to environmental variables **G. Power**

Atlantic salmon (*Salmo salar*) stocks and management implications in the Canadian Atlantic Provinces and New England, USA **R. L. Saunders**

Biological criteria for definition of species and distinct intraspecific populations of anadromous salmonids under the U.S. Endangered Species Act of 1973 **F. M. Utter**

Changes in the average size and average age of Pacific salmon **W. E. Ricker**

The stock concept and its application to British Columbia salmon fisheries **J. McDonald**

Significance of the stock concept in artificial propagation of salmonids in Alaska **J. H. Helle**

Comparative survival and growth of planted wild, hybrid, and domestic strains of brook trout (*Salvelinus fontinalis*) in Ontario lakes **J. M. Fraser**

Survival, growth, spawning distribution, and movements of introduced and native lake trout (*Salvelinus namaycush*) in two inland Ontario lakes **J. A. MacLean, D. O. Evans, N. V. Martin, and R. L. DesJardine**

Performance of indigenous, exotic, and hybrid strains of brook trout (*Salvelinus fontinalis*) in waters of the Adirondack Mountains, New York **D. A. Webster and W. A. Flick**

Structure and affinities of freshwater sea lamprey (*Petromyzon marinus*) populations **P. F. Brussard, M. C. Hall, and J. Wright**

Management implications for different genetic stocks of largemouth bass (*Micropterus salmoides*) in the United States **D. P. Philipp, W. F. Childers, and G. S. Whitt**

Native lake trout (*Salvelinus namaycush*) stocks in the Canadian waters of Lake Superior prior to 1955 **J. L. Goodier**

Survey of the genetic variation among Eastern Lake Superior lake trout (*Salvelinus namaycush*) **T. R. Dehring, A. F. Brown, C. H. Daugherty, and S. R. Phelps**

Historical evidence for discrete stocks of lake trout (*Salvelinus namaycush*) in Lake Michigan **E. H. Brown Jr., G. W. Eck, N. R. Foster, R. M. Horrall, and C. E. Coberly**

Life history and timing of migrations and spawning behavior of rainbow trout (*Salmo gairdneri*) populations of the Great Lakes **R. M. Biette, D. P. Dodge, R. L. Hassinger, and T. M. Stauffer**

Lake whitefish (*Coregonus clupeaformis*) stocks of the Ontario waters of Lake Huron
**J. M. Casselman, J. J. Collins, E. J. Crossman, P. E. Ihssen, and G. R. Spangler**
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**T. N. Todd**
Variation among stocks of walleye (*Stizostedion vitreum vitreum*): management implications
**P. J. Colby and S. J. Nepszy**
Genetic identification of sea lamprey (*Petromyzon marinus*) populations from the Lake Superior Basin **C. C. Krueger and G. R. Spangler**
Stock identification: materials and methods
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Community consequences of fish stock diversity
**R. A. Ryder, S. R. Kerr, W. W. Taylor, and P. A. Larkin**
Guidelines for the culture of fish stocks for resource management
**J. D. Hynes, E. H. Brown, J. H. Helle, N. Ryman, and D. A. Webster**
Genetic aspects of fisheries rehabilitation programs
**C. C. Krueger, A. J. Gharrett, T. R. Dehring, and F. W. Allendorf**
A wild salmon, trout, char watch: an international strategy for salmonid conservation
**P. S. Maitland, H. A. Regier, G. Power, and N. A. Nilsson**
The stock concept, discreteness of fish stocks, and fisheries management
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Perspectives and policy recommendations on the relevance of the stock concept to fishery management **G. R. Spangler, A. H. Berst, and J. F. Koonce**
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